MOLECULAR STRUCTURAL, PHYSIOCHEMICAL AND NUTRITIONAL CHARACTERIZATION OF NEW LINES OF BRASSICA CARINATA AND THE CO-PRODUCTS

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By

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ABSTRACT

Brassica carinata is widely used in biofuel industry recently because of its high oil content and good resistance. Carinata seeds contain 33% oil, 34% protein, 8% oleic acid (total fatty acids basis), 42% erucic acid (total fatty acids basis), 16% linoleic acid and 13% linolenic acid (total fatty acids basis), and 120 μmol/g glucosinolates. The co-product after oil extraction, carinata meal, is high in protein and low in fiber content. However, the molecular structural, nutritional and metabolic characteristics of yellow and brown carinata seeds of newly developed *Brassica carinata* lines from Agriculture and Agri-Food Canada (AAFC) and carinata co-products as feed ingredients are lacking. The objectives of this research were to investigate: 1) the nutritional and digestive characteristics of carinata seeds and carinata co-products for dairy cattle, 2) the molecular structural features of carinata seeds and carinata co-products, and 3) the relationship of molecular structural features to nutritional bioavailability. Yellow and brown seeds of new carinata lines were collected and compared to canola seeds from newly bred lines and a commercial cultivar. Carinata co-products, carinata meal and hexane-extracted carinata presscake, were compared with canola meal. Chemical profiles, energy values, rumen degradation kinetics of nutrients and intestinal digestion of protein were investigated, then truly absorbed protein supply to dairy cattle was predicted based on the DVE/OEB system and the NRC Dairy model. The molecular structural spectral characteristics were detected by the Fourier Transform Infrared (FTIR) vibrational spectroscopy for protein and carbohydrate related functional groups. Lastly, the relationship between nutritional values and molecular structural spectral parameters was revealed by correlation and regression studies. The results showed: 1) carinata seeds and the co-products were lower in fiber content but higher in protein; 2) carinata seeds had higher rumen degraded protein and metabolizable protein supply in dairy cows compared with canola seeds; the two carinata co-

products had higher rumen degraded protein than canola meal, but had lower intestinal digested protein; 3) both carinata seeds and co-products were higher in glucosinolates, most of which was allyl glucosinolate; 4) the hexane-extracted carinata presscake in this study had higher energy value, but showed lower intestinal absorbed protein supply to dairy cattle compared with carinata meal; 5) there were significant protein and carbohydrate structural differences among carinata and canola seeds, and among the three co-products; and 6) protein and carbohydrate structural spectral parameters had relationship with nutrient digestive features, could be used to predict nutrient bioavailability in dairy cattle.

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LIST OF ABBREVIATIONS

ABCP	Truly absorbed bypass protein in the small intestine (DVE/OEB system)
ADF	Acid detergent fiber
ADICP	Acid detergent insoluble crude protein
ADL	Acid detergent lignin
AECP	Truly absorbed rumen endogenous protein in the small intestine
AMCP	Truly absorbed microbial protein in the small intestine
ARUP	Truly absorbed rumen undegraded protein in the small intestine (NRC Dairy model)
BCP	Rumen bypass feed crude protein (DVE/OEB system)
BDNDF	Rumen bypass feed neutral detergent fiber
BOM	Rumen bypass organic matter
CA4	Sugar
CB1	Starch
CB2	Soluble fiber
CB3	Available neutral detergent fiber
CC	Unavailable neutral detergent fiber
CHO	Carbohydrate
CLA	Hierarchical cluster analysis
CP	Crude protein
D	Degradable fraction
$DE_{p3\times}$	Digestible energy at a production level (3× maintenance)
dIDP	Intestinal digestibility of rumen bypass protein on percentage basis
DM	Dry matter
DPB	Degraded protein balance

DVE	Truly digested protein in the small intestine (DVE/OEB system)
ECP	Rumen endogenous protein
ED_N	Effectively degraded nitrogen
ED_OM	Effectively degraded organic matter
EDCP	Effective degraded crude protein
EDNDF	Effective degraded neutral detergent fiber
EDOM	Effective degraded organic matter
EE	Ether extracts (crude fat)
FMV	Feed milk value
IDP	Intestinal digestible crude protein
Kd	Degradation rate of degradable fraction
Kp	Passage rate
MCP_{RDP}	Microbial protein synthesized in the rumen based on rumen degraded protein
MCP_{TDN}	Microbial protein synthesized in the rumen based on available energy (total digestible nutrients at a production level)
ME	Metabolizable energy
$ME_{p3\times}$	Metabolizable energy at a production level (3× maintenance)
MP	Metabolizable protein (NRC Dairy model)
N_MCP	Microbial protein synthesized in the rumen based on available nitrogen
NDF	Neutral detergent fiber
NDICP	Neutral detergent insoluble crude protein
NE_g	Net energy for gain
$NE_{Lp3\times}$	Net energy for lactation at a production level (3× maintenance)
NE_m	Net energy for maintenance
NFC	Non-fiber carbohydrate

NPN	Non-protein nitrogen
OEB	Degraded protein balance (DVE/OEB system)
PA2	Rapidly degradable true protein (soluble true protein)
PB1	Moderately degradable true protein
PB2	Slowly degradable true protein (bound in neutral detergent fiber)
PC	Undegradable protein
PCA	Principal component analysis
RDCA4	Ruminally degraded sugar
RDCB1	Ruminally degraded starch
RDCB2	Ruminally degraded soluble fiber
RDCB3	Ruminally degraded available neutral detergent fiber
RDPA2	Ruminally degraded rapidly-degradable true protein
RDPB1	Ruminally degraded moderately-degradable true protein
RDPB2	Ruminally degraded slowly-degradable true protein
RUCA4	Ruminally undegraded sugar
RUCB1	Ruminally undegraded starch
RUCB2	Ruminally undegraded soluble fiber
RUCB3	Ruminally undegraded available neutral detergent fiber
RUCC	Ruminally undegraded unavailable neutral detergent fiber
RUP	Rumen undegraded crude protein (NRC Dairy model)
RUPA2	Ruminally undegraded rapidly-degradable true protein
RUPB1	Ruminally undegraded moderately-degradable true protein
RUPB2	Ruminally undegraded slowly-degradable true protein
RUPC	Ruminally undegraded protein fraction
S	Soluble fraction

SCP	Soluble crude protein
T0	Lag time
tdCP	Total digestible crude protein
tdFA	Total digestible fatty acid
$TDN_{1\times}$	Total digestible nutrients at a maintenance level
tdNDF	Total digestible neutral detergent fiber
tdNFC	Truly digestible non-fiber carbohydrate
TDP	Total digestible crude protein
TRDC	Total ruminally degraded carbohydrate
TRDP	Total ruminally degraded crude protein
TRUC	Total ruminally undegraded carbohydrate
TRUP	Total ruminally undegraded crude protein
U	Rumen undegradable fraction

1. GENERAL INTRODUCTION

Brassica carinata (Ethiopian mustard) is a member of *Brassica* genus, regarded as an alternative feedstock for biofuel production. It was developed from *Brassica nigra* and *Brassica oleracea* (Hayward, 2011). Compared with canola, carinata, with good tolerance to heat and drought, is more productive and economical in semi-arid areas (Rakow and Getinet, 1998; Taylor et al., 2010; Resonance Carinata, 2012). Carinata grown in a field trial at Saskatoon, Saskatchewan (1998), contained approximately 32.6% oil, 34.1% protein, 119.8 µmol/g glucosinolates, 7.7% oleic acid (C18:1 n-9, total fatty acids basis), 42.1% erucic acid (C22:1 n-9, total fatty acids basis), 16.1% linoleic acid (C18:2 n-6, total fatty acids basis) and 13.3% linolenic acid (C18:3 n-3, total fatty acids basis) (Warwick et al., 2006). Later, the zero erucic acid *B. carinata* was developed with lower oleic acid (28.3% of total fatty acids) but higher linoleic acid (38.1% of total fatty acids) and linolenic acid (22.9% of total fatty acids) compared with *B. napus* (Getinet et al., 1994; Rakow and Getinet, 1998). Much breeding work was then conducted to increase oleic acid level in the zero erucic acid carinata (Velasco et al., 2003a; b). With higher levels of polyunsaturated fatty acids and protein, carinata seed might be suitable to be used as a feed source for animal diets. According to seed quality studies of *Brassica carinata* with different seed coat colors, yellow seeds tend to be superior as a feed source, given the higher protein content and lower dietary fiber than the brown seeds (Simbaya et al., 1995; Getinet et al., 1996).

However, carinata is higher in glucosinolates (119.8 µmol/g) compared with canola (Warwick et al., 2006). Several trials have been conducted to develop carinata lines with lower glucosinolates using genomic techniques (Getinet et al., 1997; Márquez-Lema et al., 2006; 2008). The co-product from biofuel processing, carinata meal, is commonly used in oilseed meal-based plastics or biofumigation (Galletti et al., 2008; Newson et al., 2013), because of its high

glucosinolate content. It could be considered as a feed protein source, given its high protein content, if low-glucosinolate carinata lines were developed or feed processing were conducted to reduce glucosinolates.

At present, there is no published study showing the nutritive value of recently developed carinata seeds and their co-products for dairy cattle, especially the most important digestion characteristics. The active nutritive values of feed are reflected by both chemical composition and internal molecular structure, since the nutrient bioavailability and fermentation features are influenced by the internal structure. The inherent molecular structure of feed is not detectable via conventional chemical analyses because chemicals destroy the inherent structure of functional groups. Fourier transform infrared (FTIR) vibrational spectroscopy is one of the techniques that can reveal structural chemistry and environment, with the advantage for detecting molecular structure and structural changes of various feedstuffs with different treatments (Jackson and Mantsch, 1995; Movasaghi et al., 2008; Yu et al., 2014). The aim of this research is to systematically study the molecular structural, physiochemical and nutritional characteristics of yellow- and brown-seeded *Brassica carinata* (AAFC new lines) and the co-products from biofuel processing in comparison with canola seeds (new lines and a commercial variety) and canola meal on: a) chemical composition; b) energy values for ruminants; c) protein and carbohydrate fractions, and their estimated ruminal degradation; d) rumen degradation kinetics and hourly effective degradation ratios; e) intestinal digestion of protein; f) predicted truly absorbed protein supply to dairy cattle; and g) internal protein and carbohydrate structural spectral features detected by FTIR spectroscopy. The relationship between molecular structural spectral features and nutrient bioavailability of newly developed carinata seeds and the co-products for dairy cattle is investigated to assist further development of feed nutrient analysis.

2. LITERATURE REVIEW

2.1. Carinata Development in Canada

2.1.1. Development and Production of Brassica Carinata

Brassica carinata (also called Ethiopian mustard), thought to originate from Ethiopia and other areas of East Africa, was developed from a hybridization between *Brassica nigra* and *Brassica oleracea* (Rakow, 2004; Warwick et al., 2006; Hayward, 2011). With a large demand for vegetable-based biofuel to partially replace fossil fuel globally, developing a profitable oil crop for areas with climate limitations, such as semi-arid areas, is essential. *Brassica carinata*, which has been developed by Agriculture and Agri-Food Canada (AAFC) since the mid-1990s, meets the growth requirements in the dry prairies of Western Canada (Alberta, Saskatchewan, Manitoba) and the northern plains of the USA (North and South Dakota). It offers producers high yields with high oil content in these areas regardless of heat and drought, as well as shows good salinity tolerance and blackleg resistance. It therefore helps to "save" the land dedicated to food sources (such as canola) and to address the "food vs. fuel" issue.

Currently, there are two varieties of carinata available in Canada, both of which were bred by AAFC. AAC A100 was released in 2012, and small quantities of AAC A110 were available one year later. The commercial variety of AAC A110 is now available (2015). AAC A110 has higher potential yield compared with AAC A100, with similar oil profiles (Resonance Carinata, 2012; 2015). Table 2.1 shows the yields of AAC A100 and AAC A110 collected from 2011 to 2014.

Table 2.1 Yields of *Brassica carinata* in 2011 to 2014 (Adapted from Resonance Carinata, 2015)

Year	AAC A100 Yield (kg/hectare)	AAC A110 Yield (kg/hectare)
2011	3176	3421
2012	2121	2134
2013	2542	2975
2014	-	2511

[1] Source: Agriculture and Agri-Food Canada, Agrisoma, Manitoba Agriculture, Food and Rural Initiatives, North Dakota State University, Montana State University, South Dakota State University.

[2] Sites: Saskatchewan: Pambrun, Saskatoon, Scott, Swift Current, Vanguard; Alberta: Lethbridge, Medicine Hat, Oyen; Manitoba: Melita; North Dakota: Minot, Selby; Montana: Havre.

2.1.2. Features of Brassica Carinata

Carinata has good agronomic performance in semi-arid areas, such as good biotic and abiotic stress resistance, and good resistance to blackleg (Resonance Carinata, 2015). For nutritive values, the primary AAFC carinata seed contains about 44% oil and 28% crude protein (Resonance Carinata, 2012). However, it has high erucic acid (>30% of total fatty acids) (Velasco et al., 1998; Warwick et al., 2006), a good feature for biofuel production but harmful to human and animal health. *Brassica carinata* contains 20.4% linoleic acid (C18:2, n-6) and 17.0% linolenic acid (C18:3, n-3) (Velasco et al., 1998). With the demand for human consumption, zero erucic acid carinata lines were developed (Getinet et al., 1994). The zero erucic acid *Brassica carinata* line has less oleic acid (28% of total fatty acids) than *Brassica napus* (62% of total fatty acids), but linoleic and linolenic acid contents of carinata seed are higher than those of *B. napus* (Rakow and Getinet, 1998). Later, more breeding work was focused on increasing oleic acid content in the zero erucic acid lines (Velasco et al., 2003a; b).

Distinct from canola, which is popular for its low erucic acid and glucosinolate content, *Brassica carinata* was found high in both (Xin et al., 2014a). These anti-nutritional compounds

may inhibit the digestion and absorption of important nutrients, as well as causing the bitter taste which reduces feed intake (Shahidi and Naczk, 1992; Bell, 1993). High doses of erucic acid affect heart health but may benefit the production of bioethanol and biodiesel (Vicente et al., 2005). Currently, AAFC has bred zero erucic acid and high erucic acid accessions, and are developing the low-glucosinolate lines.

Yellow varieties of *Brassica napus* were developed and selected to improve the seed quality (with thinner hulls, higher oil and protein) through interspecific crosses (Rashid et al., 1994; Rahman, 2001; Rahman and McVetty, 2011). However, different from other *Brassica* species, of which seed coat color is controlled by the maternal parent and brown color is dominant, carinata has a dominant repressor gene (Rp) inhibiting the expression of the seed coat color gene and leading to yellow seed. No recessive gene for hull color is found in *B. carinata*, the brown seed results from the absence of the repressor gene (Rahman and McVetty, 2011). The yellow carinata seed has better quality as a food or feed source with higher protein content and lower dietary fiber than the brown seed based on quality studies (Simbaya et al., 1995; Getinet et al., 1996).

2.1.3. Carinata Utilization and Carinata Meal

The zero erucic acid carinata seeds can be used for human consumption (Getinet et al., 1994), while the high erucic acid carinata seeds are mostly utilized in biofuel production (Cardone et al., 2003; Vicente et al., 2005). Currently, carinata is well accepted as a good energy source for biofuel and bio-industrial oil production, not only because of its good tolerance to drought and heat, but also because it can be easily processed by conventional crush infrastructure with minimal refining once crushed and filtered (Edwards et al., 2011). Figure 2.1 shows the biofuel processing procedures of oilseeds. Oil is extracted from the seeds and separated from the co-product (carinata meal), then refined and esterified and finally converted to biodiesel or biofuel.

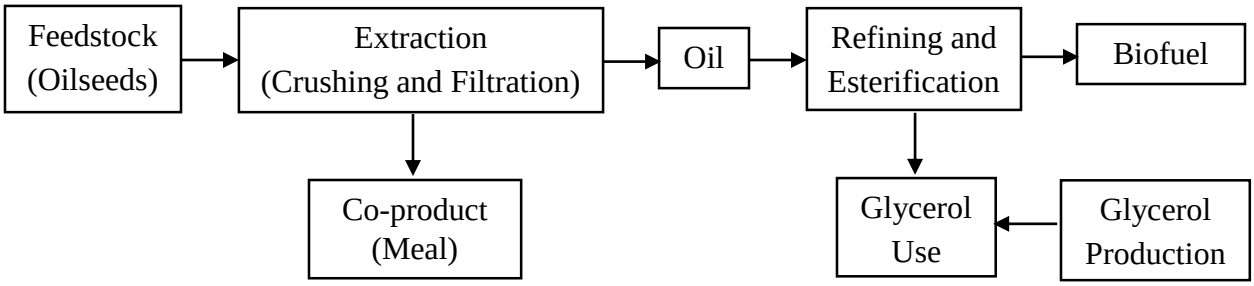


Figure 2.1 Bio-fuel processing (Adapted from Belanger, 2011; D'Avino et al., 2015)

Due to the rapid development of biofuel industry and increased utilization of carinata in Canada and USA, a large amount of carinata meal is produced during biofuel processing. Canola meal has been widely used in the animal feed industry, both for monogastric animals (Blair et al., 1986; Hilton and Slinger, 1986; Newkirk, 2009; Zhou et al., 2013) and ruminants (McKinnon et al., 1991; Piepenbrink and Schingoethe, 1998; Newkirk, 2009; Huhtanen et al., 2011) as a high protein source. However, carinata meal is not considered edible but used in oilseed meal-based plastics production and biofumigation, given its anti-nutrients (such as glucosinolates) (Galletti et al., 2008; Newson et al., 2013; D'Avino et al., 2015). Considering its high crude protein content (48%) (Xin and Yu, 2013a), carinata meal could be processed to decrease glucosinolates and then utilized in the animal feed industry.

Canadian Food Inspection Agency (CFIA) approved the use of carinata meal in beef cattle ration in 2014 (Heppner, 2014), but there is still no published research concerning the nutritional and metabolic effects of new AAFC carinata seeds and carinata co-products for dairy cows, and these carinata products are not registered in CFIA for animal feed.

2.2 Utilization and Benefits of Carinata Seeds and Co-products for Animal Feed

2.2.1. Potential Nutritional Effects of Carinata Seed

Oilseeds, such as canola with approximately 40% crude fat and 20% crude protein, have been used as a supplemental lipid and protein source in animal diets for many years (Khorasani et al., 1991; Chichlowski et al., 2005; Leupp et al., 2006). Canola contains approximately 61.4% oleic acid, 19.4% linoleic acid and 9.8% linolenic acid, while carinata contains 13.0% oleic acid, 19.9% linoleic acid and 10.8% linolenic acid (Table 2.2). It is reported that adding fat over 4% to a low quality forage-based diet will decrease nutrient digestibility (OM, CP) (Moore et al., 1986); however, feeding canola seeds to lactating dairy cattle would increase the portions of polyunsaturated fatty acids in the milk without affecting milk yield (Chichlowski et al., 2005). Some researchers found that whole canola seeds were resistant to digestion in the rumen and intestine, except when processed (Aldrich et al., 1997). There would be beneficial changes in milk fat composition when the unsaturated fatty acids in canola seeds were protected in ruminal digestion (Ashes et al., 1992). As with canola, carinata has high crude fat and crude protein, and is rich in unsaturated fatty acids. The zero erucic acid variety could be utilized in animal feed by removing glucosinolate, however the nutritive value and bioavailability of carinata seed need to be investigated for animals.

2.2.2. Potential Nutritional Effects of Carinata Meal

Canola meal (or rapeseed meal with low glucosinolates and erucic acid) is the second most commonly used protein ingredient in the world ranking after soybean meal. It is usually separated from canola oil by solvent extraction. The price of canola meal is competitive in the market compared with soybean meal, however, canola meal is lower in gross energy and protein but higher in fiber (Bell, 1993). Canola meal has good nutritive value for animals (high methionine and

cysteine), and can be widely used in poultry, swine, beef cattle, dairy cows, and fish diets (Newkirk, 2009). High in protein, carinata meal could potentially be a suitable protein source for animals as canola meal. The application of carinata meal from Agrisoma Biosciences Inc. has been approved for the use in beef cattle rations within Canada; however, there is still no published data indicating the nutritional and metabolic features of carinata meal for other animals. According to amino acid composition in defatted Brassica carinata meal compared with canola meal (Table 2.3), it is rich in arginine (10.8% of CP), glutamic acid (20.7% of CP) and proline (6.5% of CP), but lower in isoleucine (4.1% of CP), leucine (6.8% of CP), valine (4.9% of CP) and tyrosine (2.5% of CP). Pedroche et al. (2004) found carinata meal had 1.8% methionine and 2.0% cysteine (CP basis), which were lower than those of canola meal (methionine: 2.1%; cysteine: 2.4%) (Newkirk, 2009). Currently, there was no previous work on amino acid digestibility of carinata meal with dairy cows.

Table 2.2 Fatty acid composition of *Brassica carinata* in comparison with canola (a "double-zero" rape) (Adapted from Mnzava and Olsson (1990))

Fatty acids (% of total fatty acids)	*Brassica Carinata*	Canola
Palmitic	3.2	3.1
Palmitoleic	0.2	0.3
Stearic	0.9	1.1
Oleic	13.0	61.4
Linoleic	19.9	19.4
Linolenic	10.8	9.8
Arachidic	0.6	0.4
Eicosenoic	8.6	2.0
Eicosadienoic	0.8	0.1
Behenic	0.2	trace
Erucic	40.6	2.4
Docosadienoic	1.3	trace

Table 2.3 Amino acid composition of defatted carinata meal and canola meal (a "double-zero" rape) (Adapted from Mnzava and Olsson (1990), Pedroche et al. (2004); Newkirk (2009))

Amino acid (% CP)	Carinata Meal	Canola Meal	Difference
Indispensable			
Arginine	10.8	7.6	3.2
Histidine	2.9	2.9	0
Isoleucine	4.1	4.4	-0.3
Leucine	6.8	7.3	-0.5
Lysine	4.3	5.1	-0.8
Phenylalanine	3.9	4.1	-0.2
Threonine	3.9	4.6	-0.7
Valine	4.9	5.6	-0.7
Methionine	1.8	2.1	-0.3
Cysteine	2.0	2.4	-0.3
Dispensable			
Alanine	3.8	4.3	-0.5
Aspartic acid	6.6	8.1	-1.5
Glutamic acid	20.7	17.9	2.8
Glycine	4.8	5.2	-0.4
Proline	6.5	6.1	0.4
Serine	2.5	4.9	-2.4
Tyrosine	2.5	3.0	-0.5

Note: Crude protein contents in carinata meal and canola meal were 47.6 %DM and 38.7 %DM respectively (Mnzava and Olsson, 1990).

2.2.3. Cold Presscake as Animal Feed

Solvent extraction (hexane) is commonly applied in the biofuel industries for oilseed crops, such as canola, with seed conditioning, flaking and cooking as pre-treatment. To avoid cooking and save energy, cold pressing is utilized without solvent and heat, which results in higher residual oil (Seneviratne et al., 2011). The cold presscake may be a potential alternative feed source to traditional meal, however nutritional quality of carinata presscake has not been defined. McKinnon and Walker (2009) found canola presscake and mustard presscake had similar disappearance rates of dry matter (DM), crude protein (CP) and neutral detergent fiber (NDF) to canola meal for growing cattle, but their solubility and effective degradability of CP and NDF were higher. Theodoridou and Yu (2013c) also found presscake from brown canola seeds had lower protein but higher oil content than canola meal, which might be regarded as a potential high-energy source for ruminants.

2.3. Conventional Feed Evaluation for Ruminants

2.3.1. Anti-nutritional Compounds Analysis

2.3.1.1. Glucosinolates Impact

Glucosinolates, a class of sulphur-containing secondary plant metabolites, are anti-nutrients in *Brassica* genus (Tripathi and Mishra, 2007). Intact glucosinolates are thought to be biologically safe, but the degradation products may be harmful to animals (Bell, 1993). Thus, high doses of glucosinolates will have especially deleterious effects on non-ruminants and young animals (Tripathi and Mishra, 2007), such as reducing feed intake, impairing growth, liver and kidney enlargement and increasing mortality (Hill, 1991; Bell, 1993; Tripathi and Mishra, 2007). Cows are more resistant to dietary glucosinolates compared with monogastric animals, but a diet containing high glucosinolates may induce iodine deficiency, feed intake and milk production, and

even cause thyroid disturbance and depress fertility, but the negative effects of glucosinolates are relative to the concentration and animal ages (Tripathi and Mishra, 2007). Sinigrin and progoitrin are two glucosinolates which would cause bitter taste of feed. Isothiocyanates are also bitter-tasted, while thiocyanates, thiourea and oxazolidithione would depress iodine utilization and then affect thyroid function.

As a result of breeding work, the glucosinolate content has dropped to under 30 μmol/g in Canadian rapeseed and canola since 1983 (Daun, 1986). However, *Brassica carinata* is still high in glucosinolates, most of which is sinigrin (Mnzava and Olsson, 1990). A diet contains less than 11 μmol/g glucosinolates is considered relatively safe for cows (Tripathi and Mishra, 2007). Various feed processing techniques can be applied to reduce glucosinolates in the feed, such as heating (Jensen et al., 1995), extrusion (Huang et al., 1995), microwaving, microbial fermentation, water or copper sulfate treatment (Tripathi and Mishra, 2007). Breeding a low-glucosinolate carinata line or reducing glucosinolates by processing is necessary if carinata seeds and meal are to be used in the feed industry.

2.3.1.2. Condensed Tannins Impact

Condensed tannins are polymers formed by the condensation of flavan, existing mainly in the hull and more in the brown seeds than in the yellow. They will react with protein, essential amino acids or digestive enzymes and especially alter protein hydrolysis (Bell, 1993; Matthäus and Angelini, 2005). Condensed tannins were found to reduce feed digestibility in poultry and thus growth and egg weights (Martin-Tanguy et al., 1977). For ruminants, condensed tannins may affect palatability, rumen metabolism and feed digestibility (Kumar and Singh, 1984). They are toxic only when consumed in high doses (more than 1% of the diet) or at approximately 20 mg/g DM for grazing animals (Kumar and Singh, 1984). There are some beneficial effects of tannins; for

example, protecting protein from rapid rumen degradation and increasing plant protein flow into the intestine (Waghorn, 2008; Jonker et al., 2011). Several treatments can reduce condensed tannins, such as water or alkali treatment, adsorbents or urea supplementation (Kumar and Singh, 1984). Brassica carinata is not high in condensed tannins according to Matthäus and Angelini (2005).

2.3.2. Energy Value Estimation

In order to meet maintenance, growth and production requirements of animals with optimal rations at the least cost, the calorie content of feed must be investigated. Gross energy, which could be determined by combustion calorimeters, is the energy released as heat when organic matter is totally oxidized to carbon dioxide and water (NRC Beef, 1996). However, gross energy does not indicate the availability of energy to animals, which is expressed as total digestible nutrients (TDN), digestible energy (DE), metabolizable energy (ME) and net energy (NE). DE is the energy from feed gross energy minus the energy loss in the feces. TDN can be estimated using formulations from NRC Dairy (2001) and NRC Beef (1996) for ruminants, consisting of truly digestible non-fiber carbohydrate (tdNFC), truly digestible crude protein (tdCP), truly digestible fatty acid (tdFA) and truly digestible neutral detergent fiber (tdNDF), which can be obtained based on chemical profiles of feed to determine TDN at a maintenance level ($TDN_{1\times}$) according to NRC Dairy (2001). A "discount factor" is used to determine TDN at three times maintenance ($TDN_{3\times}$) based on $TDN_{1\times}$ of the diet, and further applied to DE at a production level (3× maintenance).

Metabolizable energy (ME) is the energy from DE minus urinary energy and gaseous energy, which is assumed as 0.82 times DE (NRC Beef, 1996). In dairy cattle, net energy can be separated into maintenance, growth and lactation (NE_m, NE_g and NE_L). NE_m and NE_g are the

energy used for physiological and growth performance, and can be estimated from ME (NRC Beef, 1996). NE_L is estimated at a production level of intake (3× maintenance) for lactating cows.

2.3.3. Updated Cornell Net Carbohydrate and Protein System for Feed Evaluation

The Cornell Net Carbohydrate and Protein System (CNCPS) was first published in 1992 (Fox et al., 1992; Russell et al., 1992; Sniffen et al., 1992; O'Connor et al., 1993). It was regarded as an accurate mathematical tool to estimate rumen degradation, passage and intestinal digestion of feed protein and carbohydrate and to determine nutrient requirements and animal performance (Russell et al., 1992; Fox et al., 2004; Tylutki et al., 2008). Afterwards, several updated versions have been released by the team in Cornell University. This system has fulfilled the implementation in the industry for diet formulation, such as AMTS (Agricultural Modeling and Training Systems LLC, Groton, NY) and NDS (Ruminant Management & Nutrition, Reggio Emilia, Italy). Protein and carbohydrate fractions in CNCPS are partitioned according to rumen degradation rates, and can be used to estimate feed fermentation, passage and intestinal digestion, nutrient utilization and excretion (Tylutki et al., 2008).

In CNCPS v6.1 (Tylutki et al., 2008; Van Amburgh et al., 2010), protein is partitioned to PA (NPN), PB (true protein) and PC (unavailable protein) based on rumen degradation features. PA fraction degrades at 200%/h. The true protein fraction is divided into PB1 (soluble true protein), PB2 (moderately degradable protein) and PB3 (slowly degradable protein, bound in NDF). The PB1 fraction is true protein with a rapid degradation rate of 10-40%/h, PB2 is at 3-20%/h and PB3 is at 4-9%/h for forages (the same with CHO CB3). Carbohydrate fractions include CA1, CA2, CA3, CA4, CB1, CB2, CB3, and CC, depending on rumen fermentation and microbial influence on carbohydrate availability. The CA1 fraction is volatile fatty acids (VFA) including acetate, propionate, and butyrate with 0% degradation. The CA2 fraction is lactic acid with a degradation

rate of 7%/h. The CA3 fraction contains other organic acids which degrade at 5%/h. The CA4 fraction is sugar with a degradation rate of 40 to 60%/h. The CB1 fraction is starch which degrades at 20-40%/h. CB2 fraction is known as soluble fiber with a degradation rate of 20-40%/h. The CB3 fraction is available NDF and degrades at 4 to 9%/h. The unavailable fraction (CC) is mostly cell walls (containing lignin) and non-degradable by animals.

Later, Van Amburgh et al. (2013) published CNCPS v6.5 with the update of protein partitioning and improving the prediction of amino acid requirements and supply. PA fraction (NPN) was re-characterized as PA1 (ammonia) and PA2 (soluble non-ammonia CP) (Higgs et al., 2012; Van Amburgh et al., 2013). Therefore, PB fraction is partitioned to PB1 (moderately degradable CP) and PB2 (slowly degradable CP). PC fraction is still unavailable CP. The PA1 pool maintains a degradation rate of 200%, and PA2 at 10-40%/h. PB1 has the degradation rate of 3-20%/h, and PB2's degradation rate is the same with that of CB3 adjusted from 4-9%/h to 1-18%/h (Van Amburgh et al., 2015). Amino acid profiles were updated together with the adoption of a combined efficiency of essential amino acid utilization for maintenance and lactation in this revision. The complete update to CNCPS v6.5 was released in 2015 (Higgs et al., 2015; Van Amburgh et al., 2015). The partition of CHO is not changed; however unavailable NDF (CC fraction) changes from the estimated value ((lignin×2.4)/NDF) to the determined value based on 240 h *in vitro* digestibility (Raffrenato, 2011). The adjusted chemical methods are pointed out in Higgs et al. (2015). The NDF analysis should be corrected based on organic matter (NDF_{OM}).

2.3.4. In Situ Technique for Determining Rumen Degradation Kinetics of Feed Nutrients

Dietary nutrient bioavailability is affected by rumen degradation and fermentation. Feed crude protein (true protein and NPN) is broken down in the rumen by microorganisms and used to synthesize microbial protein for milk production (Satter and Roffler, 1975). Digestion of organic

matter provides the energy for microbial protein synthesis. Rumen degradation characteristics reflect nutritional values of feed ingredients. The in situ technique (or in situ nylon bag technique) is a simple and efficient way to explore degradation characteristics of feed inside the rumen. This approach in 1930s featured silk bags containing feed samples in cannulated sheep (Quin et al., 1939). Since then, the in situ nylon bag technique became popular and widely adopted to estimate feed ruminal degradation kinetics (Ørskov et al., 1980), especially of protein (Ørskov and McDonald, 1979). It is a rapid way to estimate feed degradation and allows handling several samples together at once. Although there are other techniques to detect rumen degradation characteristics, the in situ technique is regarded as the better method, even though it is affected by microbial activity, bag porosity, feed particle size and individual animal differences (Nocek, 1988). Nutrient ruminal degradation is measured by the disappearance from bags into the rumen. The first order kinetic nonlinear model was described by Ørskov and McDonald (1979) with degradation parameters and incubation time. Subsequently, this model was improved by adding "lag time (T0)" (Robinson et al., 1986; Dhanoa, 1988), resulting in greater accuracy:

$$R(t) = U + (100 - S - U) \times e^{-Kd \times (t - T0)}$$

Where, R(t) is the residue after t h incubation (%), U is undegradable fraction (%), S is soluble fraction from 0 h incubation (%), Kd is degradation rate (%/h) and T0 is the lag time (h).

Microbial protein synthesis is influenced by protein content and energy supply, and a reflection of the balance between nitrogen and energy supply. Hourly effective degradation of nutrients can be approximated by applying incubation time intervals into the equation (Sinclair et al., 1993; Nuez-Ortín and Yu, 2010).

$$\text{Hourly ED} = S + [(D \times Kd) / (Kp + Kd)] \times [1 - e^{-t \times (Kd + Kp)}]$$

$$\text{Hourly ED N/OM}_t = (\text{HEDN}_t - \text{HEDN}_{t-1}) / (\text{HEDOM}_t - \text{HEDOM}_{t-1})$$

Where, S is the soluble fraction (%), D is potentially degradable fraction (%), Kd is degradation rate (%/h), Kp is the passage rate (%/h), N/OM_t is the ratio of N to OM at time t (g N/kg OM), $HEDN_t$ is effective degradability of N at time t (g/kg DM), $HEDN_{t-1}$ is effective degradability of N at 1 h before time t (g/kg DM), $HEDOM_t$ is effective degradability of OM at time t (g/kg DM), and $HEDOM_{t-1}$ is effective degradability of OM at 1 h before time t (g/kg DM). According to previous studies (Czerkawski, 1986; Tamminga et al., 1990; Sinclair et al., 1993), the optimal effective degradation ratios are assumed to be 25 g N/kg OM degraded in the rumen.

2.3.5. A Three-step In Vitro Technique for Estimating Intestinal Digestion of Protein

The protein available for absorption, consisting of microbial protein and dietary nitrogen, passes from the rumen to the small intestine. Several methods have been used to estimate the intestinal digestibility of available N. Using intestinally-cannulated animals is considered costly and difficult to maintain. In vitro methods to replace the in vivo method should involve similar physiological conditions and be accurate for various feed sources. A three-step in vitro procedure was developed to estimate protein digestion in the small intestine, and is considered a rapid and accurate method meeting these requirements (Calsamiglia and Stern, 1995). This technique has been adopted by NRC Dairy (2001) as a reference method. Using this method, residuals with 15mg N after rumen incubation of 16 hours are incubated in 10 mL 0.1 mol/L HCl solution containing pepsin (pH = 1.9) at 38 ºC for 1 hour in a shaking water bath and then neutralized using 0.5 mL 1 mol/L NaOH and 13.5 mL phosphate buffer containing pancreatin (pH = 7.8). The mixture is incubated for 24 h at 38 ºC in the shaking water bath, and vortexed every 8 h. Three mL trichloroacetic acid (TCA) solution is then added into the mixture to stop enzymatic hydrolysis and precipitate undigested protein. After vortexing and sitting for 15 minutes, samples are centrifuged and the supernatant is analyzed for soluble N using the Kjeldahl method.

2.3.6. Prediction of Truly Absorbed Protein Supply to the Small Intestine of Dairy Cattle

The dietary protein is the only a part of truly utilized protein which is absorbed and digested in the small intestine. To predict true protein utilization in the small intestine, various mathematic models have been developed, such as the PDI system (Vérité and Geay, 1987), the DVE/OEB system (Tamminga et al., 1994) and the NRC model (NRC Dairy, 2001). The DVE/OEB system and NRC Dairy model are two modern systems used to estimate dietary protein supply to dairy cattle for milk production. The DVE/OEB system was established by Tamminga et al. (1994) and widely accepted in some European countries. This system was updated in 2010 (Van Duinkerken et al., 2011). The latest NRC Dairy model was published in 2001 by National Academies Press (NAP). Several studies have been conducted to compare the two systems with various feeds in order to distinguish the differences (Yu et al., 2003; Theodoridou and Yu, 2013b).

2.3.6.1. DVE/OEB System

The DVE/OEB system was first developed based on the PDI system (Tamminga et al., 1994). In this system, the supply of true protein to the small intestine is considered as true protein digested in the small intestine (DVE), which consists of ruminal undegraded feed protein absorbed in the small intestine (DVBE) and microbial protein absorbed from the small intestine (DVME). However, the DVE value is predicted from the sum of DVBE and DVME minus endogenous protein losses during digestion (DVMFE). This system allows more accurate feed protein supply predictions and prevention of N losses. A balance between true energy and N supply from feed is essential to maximize microbial protein synthesis. In this system, the degraded protein balance (OEB) of feed indicates the difference between total microbial protein synthesis from rumen degraded feed protein and that providing energy to rumen fermentation. A positive OEB value indicates potential loss of N from the rumen, while a negative value represents a shortage of N

supply, resulting in impaired protein synthesis. To maximize N utilization, a zero or slightly above OEB value is recommended. DVE and OEB values of feed ingredients are used for dairy ration formulation to meet protein requirements in different stages.

An updated DVE/OEB_{2010} system was developed after the primary DVE/OEB_{1994} system (Tamminga et al., 2007; Van Duinkerken et al., 2011). In this latest system, the fractions based on in situ rumen degradation have been changed to washable soluble fraction (W), washable insoluble fraction (WI), nonwashable degradable fraction (D) and nonwashable undegradable fraction (U). The estimation of microbial protein synthesis considers contributions from additional components (CP, NDF, starch, sugar, nonstarch polysaccharides) and their in situ degradable fractions. Therefore, the estimation of DVE value in DVE/OEB_{2010} is totally different from DVE/OEB_{1994}.

2.3.6.2. NRC Dairy Model

The NRC Dairy model predicts the truly digested and absorbed protein in the small intestine. However, unlike the DVE/OEB system, the NRC Dairy model is based on the TDN value, and the endogenous protein from the rumen (ECP) is considered to contribute to the metabolizable protein (MP). According to NRC Dairy (2001) and Theodoridou and Yu (2013b), MP is calculated as the sum of truly absorbed rumen undegraded feed protein in the small intestine (ARUP), truly absorbed microbial protein in the small intestine (AMCP), and the truly absorbed endogenous protein in the small intestine (AECP). The concept of degraded protein balance in DVE/OEB system (Tamminga et al., 1994; 2007) is accepted and can be applied in the NRC model, and calculated as potential synthesized microbial protein based on ruminally available feed protein (RDP) minus microbial protein synthesized based on TDN as available energy.

2.4. Mid-Infrared Vibrational Spectroscopy in Feed Analysis

2.4.1. Infrared Spectroscopy

The nutritive values of feed ingredients are influenced not only by chemical composition but also by inherent molecular structure, while considering the nutrient bioavailability and fermentation features (Yu, 2012). However, molecular structure of feed cannot be detected by "wet" chemical analyses because chemicals and lab digestion will destroy the internal structure of functional groups. Infrared spectroscopy is one technique identifying molecular-level information. Infrared radiation (IR), with frequencies between 14000 and 4 cm^{-1}, consists of near IR (ca. 14,000-4,000 cm^{-1}), mid IR (ca. 4,000-400 cm^{-1}) and far IR (ca. 400-4 cm^{-1}) (Smith, 2011). The fundamental principle of infrared spectroscopy is the vibrations of atoms. The molecule specific spectral bands provide information about biochemical composition (Movasaghi et al., 2008). When infrared radiation passes through a sample, functional groups in the sample will absorb part of the radiation at a specific frequency. The absorbed energy appears as a peak in the spectrum and corresponds to the vibration frequency of the molecule (Stuart, 2005). Infrared spectroscopy measures infrared frequencies absorbed by various bands, and can detect the differences among samples by comparing spectral parameters.

2.4.2. Fourier Transform Infrared Vibrational Spectroscopy

2.4.2.1. Basic Principles

Fourier transform infrared spectroscopy with attenuated total reflectance (FTIR-ATR) consists of three fundamental spectrometer components: the radiation source, the interferometer (a moving mirror, a fixed mirror and a beamsplitter) and the detector as Figure 2.2 shows (Hsu, 1997; Smith, 2011). A beam of infrared light from the radiation source is collimated and directed to the beamsplitter, where the beam is divided into two parts. One part is transmitted to the fixed mirror goes back and the other part reflects off the moving mirror. Finally, both beams are combined at the beamsplitter and pass through the sample. Part of beam will be absorbed by the

sample, and the rest will be collected by a detector behind the sample, which shows the attenuated intensity of the total reflected infrared beam minus the beam absorbed by the sample on a spectrum (Smith, 2011).

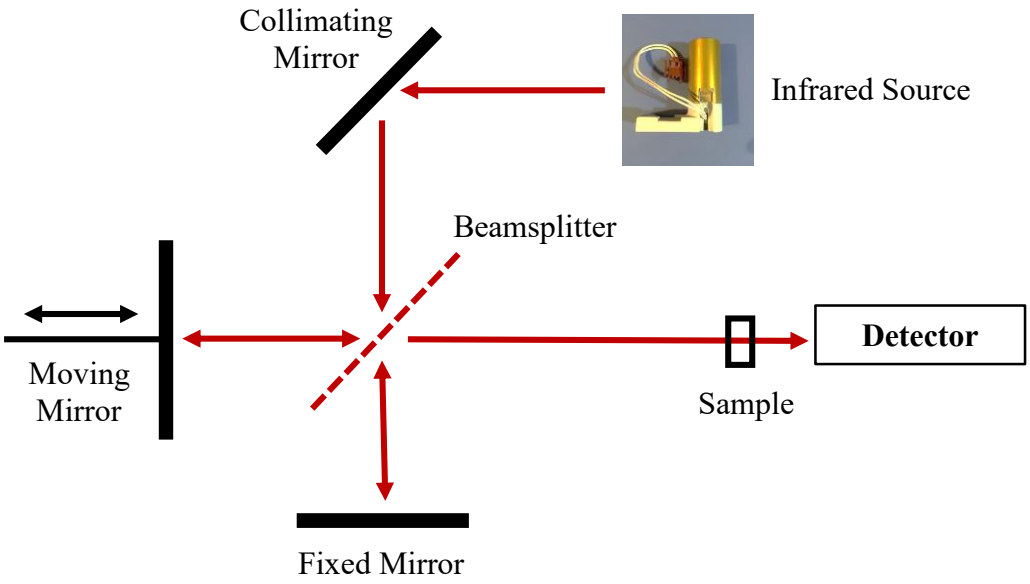


Figure 2.2 Fourier transform infrared (FTIR) vibrational spectroscopy (Adapted from Smith, 2011)

2.4.2.2. Application of FTIR in Feed Analysis

FTIR is a rapid and accurate spectroscopic technique for analyses of samples in liquid, solid and gas states. It has been applied in fields of physics, chemistry, biology as well as agriculture (Smith, 2011). It is universal because many molecules have strong absorbance in the mid-infrared region, where spectra are commonly measured. With the high sensitivity, only small amounts of samples are required with little sample preparation. Moreover, it is relatively inexpensive with mass information revealed in the spectra, such as molecule chemical matrix, chemical concentrations and chemical environment.

Recently in feed science, it has been successfully used to detect structural changes of molecules and conformation of biopolymers among different kinds of feedstuff in relation to nutrient values, nutrient utilization and availability. For example, FTIR-ATR are used to detect the molecular structural differences among feed-based crop varieties, feed ingredients, effects of gene modification and feed processing on spectral characteristics, and effects of rumen degradation on protein- and carbohydrate-related structures (Damiran and Yu, 2011; Abeysekara and Yu, 2012; Theodoridou and Yu, 2013a; Xin and Yu, 2013b; Li et al., 2015).

2.4.2.3. Spectral Analysis Methods

Univariate and multivariate analyses are the two statistical methods applied to interpret spectral features of feedstuff based on infrared spectroscopic techniques (Yu, 2005a). In the univariate analysis, mathematical parameters in spectra are obtained including band height and area intensities, band frequencies and the band intensity ratios. The univariate analysis relates the spectral intensity information to the biological meaning. However, because it is difficult to analyze and compare massive spectral data with the univariate method, multivariate analysis is preferred. Hierarchical cluster analysis (CLA) and principal component analysis (PCA) are two widely-used methods (Yu, 2005a). The CLA method groups samples similar in spectral data and presents results as dendrograms with distance matrix. A new "hierarchical group" (called "cluster") is obtained by combining two of the most similar spectra (minimal distance), and then distances between all remaining spectra are recalculated to create a tree diagram. In the PCA method, the original spectral data with interrelated variables is transformed to a new dataset with uncorrelated variables called "principal components" (PCs), of which the first few may account for more than 95% variance (Martin et al., 2004). The analyses results are usually reflected by two dimensional (two PCs) or three dimensional (three PCs) scatter plots.

2.5. Literature Summary, Research Hypotheses and Objectives

Brassica carinata is a productive oilseed crop for biofuel production in dry prairie areas including Western Canada and Northern USA. It is high in protein and oil content, and includes varieties with low erucic acid and high erucic acid. Both carinata seed and carinata meal have high amounts of glucosinolates, which may produce anti-nutritional effects in humans and animals. These anti-nutritional effects may be altered via feed processing and breeding. Similar to canola meal, carinata meal is high in protein (approximately 48%), which makes it a potential feed protein source. However, with little research about the nutritional values of carinata seeds and carinata co-products from biofuel processing, the metabolic consequences are as yet unidentified for animals.

Currently, there are yellow-seeded and brown-seeded *Brassica carinata* lines developed by Agriculture and Agri-food Canada (AAFC). Based on previous studies, yellow seed has higher oil and protein contents, and lower crude fiber compared to brown seeds (Getinet et al., 1996), whereas the differences in degradation and digestion features are not clear. Hence, this project was designed to investigate the molecular structural, physiochemical and nutritional characteristics of newly developed yellow and brown carinata seeds (AAFC new lines) and carinata co-products in comparison with canola seeds (AAFC new lines and a commercial variety) and meal. Chemical composition, nutrient bioavailability and utilization, as well as protein and carbohydrate structure of carinata seeds and the biofuel co-products will be evaluated to identify the nutritional values for dairy cattle and the relationship between structural and metabolic features.

2.5.1. Hypotheses

The hypotheses of this study are:

- Carinata seeds (new lines) have significantly different molecular structural and nutritional features compared with those of canola seeds (new lines and the variety), thus resulting in different nutrient supply to dairy cattle.
- Carinata meal has significant differences from canola meal in nutritional characteristics, nutrient supply and structural spectral features. The hexane-extracted carinata presscake may show differences in inherent structure, nutrient degradability and digestibility, therefore providing a unique nutrient supply and bioavailability for dairy cattle.

2.5.2. Objectives

The objectives of this study are:

- To systematically study molecular structural, physiochemical and nutritional features of newly developed yellow and brown carinata seeds (AAFC new lines) as well as carinata co-products compared with canola seeds (AAFC new lines and a commercial variety) and canola meal in terms of a) chemical profiles and anti-nutritional compounds; b) energy values for ruminants; c) protein and carbohydrate fractions, and their estimated rumen degradation; d) rumen degradation kinetics of nutrients; e) intestinal digestion of protein; f) predicted truly absorbed protein supply to dairy cattle; and g) inherent molecular structure spectral characteristics detected by the FTIR technique.
- To explore the relationship between inherent structure spectral features and nutrient bioavailability of new carinata seeds and carinata co-products for dairy cattle.
- To assist further development and utilization of new carinata seeds and carinata co-products for feed industry.

3. RUMINANT NUTRITION STUDY OF BRASSICA CARINATA SEEDS AND THE CO-PRODUCTS

3.1. Introduction

Brassica carinata is a substitute oilseed crop well accepted in the rapidly developing biofuel and bio-industrial oil industry (Carlsson, 2009; Taylor et al., 2010), because of its excellent agronomic characteristics in dry areas as well as good disease resistance. Carinata seeds contain about 26-40% oil and originally were high in erucic acid (Velasco et al., 1998; Warwick et al., 2006; Xin et al., 2014a), which would be ideal for biodiesel production. As reported earlier, *Brassica carinata* had 20.4% linoleic acid (fatty acid basis) and 17.0% linolenic acid (fatty acid basis) (Velasco et al., 1998). Zero erucic acid carinata varieties were developed to suit human consumption with low oleic acid (28.3% of total fatty acids) (Getinet et al., 1994). The fatty acid profile of zero erucic acid carinata seed consists of 38% linoleic acid and 23% linolenic acid (fatty acid basis), higher than those in canola seeds (Getinet et al., 1994; Rakow and Getinet, 1998). However, glucosinolates are high in carinata (119.8 μmol/g) (Warwick et al., 2006), most of which is allyl glucosinolate (sinigrin). Subsequently, breeding work was centered on modifying its seed quality. For example, increasing oleic acid in zero erucic acid varieties (Velasco et al., 2003a; b) and developing low-glucosinolate lines by genomic techniques (Getinet et al., 1997; Márquez-Lema et al., 2006; 2008). According to various quality studies of *Brassica* seeds with different seed coat colors, yellow seeds have thinner hulls with lower lignin and dietary fiber, but higher protein and oil content than the brown, thus providing higher quality meal (Simbaya et al., 1995; Getinet et al., 1996). The co-product from biofuel processing, carinata meal, have been used to produce oilseed meal-based plastics or used as a biofumigant (Galletti et al., 2008; Newson et al., 2013). However, carinata co-products may be potentially superior protein sources, given their high

protein content and low fiber, if low-glucosinolate lines were developed or modifications were conducted to reduce existing anti-nutritional compounds.

The degradation extent and rate of nutrients (dry matter, organic matter, crude protein and NDF) can be evaluated by the in situ nylon bag technique (Yu et al., 1999), and the in vitro technique is an effective way to estimate the intestinal digestion of protein (Calsamiglia and Stern, 1995). Protein absorption in the small intestine can be estimated by the DVE/OEB system and the NRC Dairy model, in order to predict the truly absorbed protein values of carinata seeds and the co-products for dairy cattle.

In order to determine the nutritional qualities of carinata seeds (AAFC new lines) and carinata co-products for potential utilization in animal feed industry, the objectives of this study were to systematically evaluate 1) chemical profiles and anti-nutritional compounds, 2) energy values, 3) protein and carbohydrate fractions, and their predicted rumen degradation, 4) rumen degradation kinetics, and hourly effective degradation ratios, 5) intestinal digestion of protein, 6) truly absorbed protein supply to dairy cows and feed milk values in comparison with canola seed (new lines and a variety) and canola meal.

3.2. Materials and Methods

3.2.1. Sample Preparation

Recently developed yellow and brown *Brassica carinata* and canola lines were provided by Agriculture and Agri-Food Canada (Saskatoon, Saskatchewan), with each line collected from two source years as replicates. A commercial canola seed (*Brassica napus*) was used for comparison (Table 3.1.1).

The co-products in this study were carinata meal and hexane-extracted carinata presscake, together with canola meal as the reference (Table 3.1.2). The oil extraction for carinata seed

(mixed-color seed) was conducted by POS Bio-Sciences (Saskatoon, SK), with oil shipped to another facility for biofuel conversion. Two sources of carinata meal were collected from Agrisoma Biosciences Inc. (Saskatoon, SK) in 2014. The processing of hexane-extracted carinata presscake was conducted in POS Bio-Sciences (Saskatoon, SK) by Agrisoma Biosciences Inc. (Saskatoon, SK). Three sources (from different sites) of mixed-colored carinata seeds (yellow and brown) were transferred into a 4 L plastic pail and weighed. Water was sprayed onto the seeds to maintain a 2.5% moisture content. After mixing, the tempered seeds were flaked using the lab flaking mill and heated in beakers in a microwave at full power for 2.5 minutes. Then all beakers were covered and transferred to a convection oven for 20 minutes at 95±3 °C. Afterwards, the cooked flakes were pressed using the Gusta Lab Press (Gusta Manufacturing, Winnipeg, MB) through a 5/16 die plate, with crude-pressed oil collected during the processing. The lab Soxhlet extractor was used to extract the oil in the presscake with 6 liters of fresh hexane for 4 hours. Lastly, the hexane-extracted carinata presscake was placed in a fume hood for a minimum of 36 hours for air desolventizing. Then three sources of hexane-extracted carinata presscake were collected and compared with carinata meal and canola meal. Two sources of canola meal from bio-oil processing were provided by Federated Cooperatives Limited (Saskatoon, SK) as a reference.

Table 3.1.1 Seed codes and sources of new yellow and brown carinata seeds, new yellow and brown canola seeds, and a commercial brown canola seed

Feed[1]	Line Code	Sample Source	Seed Coat Color
New Carinata Seed	AAC-A110	2012, 2013	Yellow
New Carinata Seed	110915EM	2012 (1, 2)	Brown
New Canola Seed	YN07-C1386	2008, 2011	Yellow
New Canola Seed	N07-1374	2010, 2011	Brown
Commercial Canola Seed	*Brassica napus*	2010, 2011	Brown

[1] All the seed samples were provided by Agriculture and Agri-Food Canada (AAFC).

Table 3.1.2 Sources and providers of carinata meal, hexane-extracted carinata presscake and canola meal

Feed	Source	Provider
Carinata Meal	Bio-fuel 1, 2	Agrisoma
Hexane-extracted Carinata Presscake	Bio-fuel 1, 2, 3	Agrisoma
Canola Meal	Bio-oil 1, 2	Federated Co-op

3.2.2. Animals and Diets

The experimental procedures used in this study was approved by the University of Saskatchewan Animal Research Ethics Board (AREB) with Animal Use Protocol # 19910012, and conducted according to the guidelines of the Canadian Council of Animal Care (1993). Four lactating rumen-cannulated Holstein cows were used to estimate the rumen degradation kinetics of carinata seeds and carinata co-products. The internal diameter of each rumen cannula (Bar Diamond, Parma, ID) was 10 cm. Cows were individually fed twice daily at 0800 and 1600 h with a total mixed ration (TMR) in a tie-stall barn at the Rayner Dairy Research and Teaching Facility (University of Saskatchewan, Saskatoon, SK), and given free access to water. The TMR was formulated with 48.5% barley silage, 12.1% hay and 31.3% concentrate according to NRC Dairy requirements (NRC Dairy, 2001).

3.2.3. Chemical Analyses and Anti-nutritional Compounds

For chemical analyses, all samples (seeds and co-products) were ground with a coffee grinder (PC770, Loblaws Inc., Toronto, ON) for 20 seconds. Dry matter (DM) (AOAC official method 930.15), ash (AOAC official method 942.05), crude fat (EE) (AOAC official method 954.02), and crude protein (CP) (AOAC official method 984.13) were analyzed in accordance with the AOAC official methods (1990). The neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) in the samples were determined using the methods described in

Van Soest et al. (1991) using the ANKOM A200 filter bag technique (ANKOM Technology Corp., Fairport, NY, US), after the oil content of oilseed samples was removed. Neutral detergent insoluble crude protein (NDICP) and acid detergent insoluble crude protein (ADICP) were analyzed using the NDF and ADF residues according to the methods in Licitra et al. (1996). To evaluate the total soluble crude protein (SCP) content, all samples were incubated in a bicarbonate-phosphate buffer, and filtered through #54 Whatman filter papers (Roe et al., 1990). Non-protein nitrogen (NPN) was estimated as the difference between total crude protein and precipitated true protein in tungstic acid (Licitra et al., 1996). Non-fiber carbohydrate (NFC) was calculated as 100 - (NDF - NDICP) - EE - CP - Ash according to NRC Dairy (2001).

For anti-nutritional compounds, glucosinolates were estimated according to the official method of the Canadian Grain Commission (Daun and McGregor, 1983) by POS Bio-Sciences (Saskatoon, SK). The condensed tannins were determined according to the HCl−butanol procedure (Porter et al., 1985).

3.2.4. Energy Values

The truly digestible non-fiber carbohydrates (tdNFC), the total digestible neutral detergent fiber (tdNDF), the total digestible crude protein (tdCP) and the total digestible fatty acid (tdFA) were estimated according to the NRC Dairy (2001) based on feed chemical composition. Subsequently, total digestible nutrients at a maintenance level ($TDN_{1\times}$), digestible energy at a production level ($DE_{p3\times}$, 3 times maintenance), metabolizable energy at a production level ($ME_{p3\times}$, 3 times maintenance), and net energy at a production level ($NE_{Lp3\times}$, 3 times maintenance) were estimated. The estimations of the metabolizable energy (ME), the net energy for maintenance (NE_m) and the net energy for gain (NE_g) were described by NRC Beef (1996) and NRC Dairy (2001).

3.2.5. Protein and Carbohydrate Fractions

In the Cornell Net Carbohydrate and Protein System (Van Amburgh et al., 2010; 2013; Higgs et al., 2012), protein is partitioned to PA (ammonia and soluble true protein), PB (moderately and slowly degradable true protein) and PC (unavailable protein) based on their rumen degradation features. The PA1 fraction (ammonia) and PA2 fraction (soluble true protein) have degradation rates 200%/h and 10-40%/h respectively. The remaining true protein is divided into the PB1 fraction (moderately degradable protein) and PB2 fraction (slowly degradable protein) with degradation rates of 3-20%/h and 4-9%/h. The PC fraction is unavailable protein without degradation.

Carbohydrate partition is described in Van Amburgh et al. (2010) and Higgs et al. (2012). The eight subfractions include CA1 (volatile fatty acids), CA2 (lactic acid), CA3 (other organic acids), CA4 (sugar), CB1 (starch), CB2 (soluble fiber), CB3 (available NDF) and CC (unavailable NDF), based on rumen fermentation and microbial activity on carbohydrate availability (Lanzas et al., 2007; Van Amburgh et al., 2010). The CA1 fraction is volatile fatty acids (VFA) consisting mainly of acetate, propionate and butyrate, which are not degradable (0%/h). The CA2 fraction is lactic acid with a degradation rate of 7%/h. The CA3 fraction includes other organic acids which degrade at 5%/h. The CA4 fraction, sugar, degrades at 40 to 60%/h. The CB1 fraction, starch, degrades at 20 to 40%/h. The CB2 fraction, soluble fiber, degrades at 20-40%/h. The CB3 fraction, available NDF, degrades at 4 to 9%/h. The unavailable fraction, CC, mostly plant cell walls containing lignin, is considered undegradable. The specific degradation rates of protein and carbohydrate fractions in oilseeds and their co-products for this study can be found in NDS software based on the CNCPS feed library (Ruminant Management & Nutrition, Reggio Emilia, Italy). The passage rate (Kp) is 12%/h for CA4 and PA1, and 6%/h for other fractions (Van Amburgh et al., 2010).

3.2.6. Rumen Degradation Kinetics of Nutrients and Hourly Effective Rumen Degradation Ratios

The in situ technique is the most effective method of study of rumen nutrient degradation kinetics. All seed and co-product samples were roughly ground with a coffee grinder (PC770, Loblaws Inc., Toronto, ON) for 10 seconds. Seven grams (7 g) of samples were weighed and placed into numbered nylon bags with 40 μm pores. All bags were tied and randomly placed into the rumens of four cannulated lactating Holstein cows for 48, 24, 12, 8, 4, 2, 0 h incubations in two runs according to a "gradual addition and all out" schedule (Yu et al., 2000). After incubation, all the bags were removed from the rumens and washed of ruminal contents in detergent-free cold water, and dried at 55°C for 48 h. The dried residues of each sample were weighed and pooled for chemical analyses based on treatments, incubation time and run. Before analyses, all the pooled residue samples were ground by the same coffee grinder (PC770, Loblaws Inc., Toronto, ON) for another 10 s. DM and ash were analyzed based on AOAC official methods (1990). Crude protein in residues was analyzed by Leco Protein/N Analyser (FP-528, Leco Corp., St. Joseph, MI, US). NDF in residues was detected according to Van Soest et al. (1991) and the ANKOM A200 filter bag technique (ANKOM Technology Corp., Fairport, NY, US), with sodium sulfite and α-amylase added. Oil content in residues of oilseed samples was removed by ether extraction before NDF analysis.

The first order kinetics degradation model with lag time was used to reveal the degradation features of OM, CP and NDF (Ørskov and McDonald, 1979; Robinson et al., 1986; Tamminga et al., 1994). The data was processed using the non-linear (NLIN) procedure of SAS 9.3 (SAS Institute, Inc., Cary, NC, US) and iterative least-squares regression (Gauss-Newton method).

$$R(t) = U + D \times e^{-Kd \times (t-T0)}$$

Where, R(t) is the residue after t h incubation (%), U is undegradable fraction (%), D is degradable fraction (%), Kd is degradation rate (%/h), and T0 is lag time (h).

The rumen undegraded (bypass) and degraded values of nutrients were estimated with passage rate (Kp) assumed as 6%/h (Tamminga et al., 1994).

$$\%BOM\ (\%BCP\ /\ \%BNDF) = U + D \times [Kp / (Kp + Kd)]$$

$$\%EDOM\ (\%EDCP\ /\ \%EDNDF) = S + D \times [Kd / (Kp + Kd)]$$

$$BOM\ (g/kg\ DM) = OM\ (g/kg\ DM) \times \%BOM$$

$$BCP^{DVE}\ (g/kg\ DM) = 1.11 \times CP\ (g/kg\ DM) \times \%BCP$$

$$RUP^{NRC}\ (g/kg\ DM) = CP\ (g/kg\ DM) \times \%BCP$$

$$BNDF\ (g/kg\ DM) = NDF\ (g/kg\ DM) \times \%BNDF$$

$$EDOM\ (EDCP\ /\ EDNDF,\ g/kg\ DM) = OM\ (CP\ /\ NDF,\ g/kg\ DM) \times \%EDOM\ (\%EDCP\ /\ \%EDNDF)$$

Where, BOM, BCP (RUP) and BNDF are rumen undegraded OM, CP and NDF respectively, ED is effective degradability, U is undegradable fraction (%), D is degradable fraction (%), S is soluble fraction (%), Kd is degradation rate (%/h), Kp is passage rate (%/h). Rumen bypass crude protein is estimated differently in the DVE/OEB system (Tamminga et al., 1994) and NRC Dairy model (NRC Dairy, 2001).

The nutrients degraded hourly can be estimated based on the equation from Sinclair et al. (1993). Based on this previous study, the equation of hourly effective degradation ratios of ED_N to ED_OM was published in Nuez-Ortín and Yu (2010).

$$\text{Hourly ED} = S + [D \times Kd / (Kp + Kd)] \times [1 - e^{-t \times (Kd + Kp)}]$$

$$\text{Hourly ED N/OM}_t = (HEDN_t - HEDN_{t-1}) / (HEDOM_t - HEDOM_{t-1})$$

Where, ED is effective degradability, S is soluble fraction (%), D is degradable fraction (%), Kd is degradation rate (%/h), Kp is passage rate (%/h), N/OM_t is the ratio of N to OM at time t (g N/kg OM), $HEDN_t$ is effective degradability of N at time t (g/kg DM), $HEDN_{t-1}$ is effective

degradability of N at 1 h before time t (g/kg DM), $HEDOM_t$ is effective degradability of OM at time t (g/kg DM), and $HEDOM_{t-1}$ is effective degradability of OM at 1 h before time t (g/kg DM).

3.2.7. Intestinal Digestion of Protein

Protein digestion in the small intestine was estimated by a modified three-step in vitro procedure (Calsamiglia and Stern, 1995; Yu et al., 2000). Fifteen grams (15 g) of residues from 12 hours in situ rumen incubation were weighed and mixed with 10 mL 0.1 mol/L HCl solution containing pepsin (pH = 1.9), and then incubated at 38 ºC for 1 hour in a shaking water bath. After incubation, they were neutralized by 0.5 mL 1 mol/L NaOH and 13.5 mL phosphate buffer containing pancreatin (pH = 7.8). Once vortexed, the mixture was incubated for 24 h at 38 ºC in the shaking water bath and vortexed every 8 h. In order to stop enzymatic action and precipitate the undigested protein, three mL (3 mL) trichloroacetic acid (TCA) solution was added to the mixture. Samples were then centrifuged at 5000 rpm for 15 min, and 5 ml of each supernatant was analyzed for soluble N by the Kjeldahl method. The soluble protein was counted as the digested protein in the small intestine.

3.2.8. Predicted Truly Absorbed Protein Supply for Dairy Cattle and Feed Milk Value

3.2.8.1. DVE/OEB System

The truly digested feed protein in the small intestine can be estimated based on chemical composition, ruminal protein degradation and intestinal protein digestion. In the DVE/OEB model (Tamminga et al., 1994), DVE equals the sum of truly absorbed rumen-undegraded feed protein in the small intestine (ARUP) and truly absorbed rumen-synthesized microbial protein in the small intestine (AMCP) minus endogenous loss during digestion (ENDP).

$$DVE\ (g/kg\ DM) = AMCP^{DVE} + ABCP^{DVE} - ENDP$$

The estimation of microbial crude protein synthesis is based on organic matter fermentation in the rumen (Theodoridou and Yu, 2013b).

$$MCP_{FOM}\ (g/kg\ DM) = 0.15 \times FOM\ (g/kg\ DM)$$

Where, 0.15 indicates that 150 g microbial crude protein is synthesized based on 1000 g rumen fermented OM (FOM).

The truly absorbed rumen-synthesized microbial protein in the small intestine (AMCP) was estimated as:

$$AMCP^{DVE}\ (g/kg\ DM) = 0.75 \times 0.85 \times MCP_{FOM}\ (g/kg\ DM)$$

Where, 0.75 indicates 75% of microbial crude protein present as amino acids, and 0.85 is the efficiency of microbial protein digestion in the small intestine.

Secondly, truly absorbed rumen-undegraded feed protein in the small intestine (ARUP) was estimated based on the results of in vitro intestinal protein digestion of RUP as below:

$$ABCP^{DVE}\ (g/kg\ DM) = [dIDP\ (\%) \times BCP^{DVE}\ (g/kg\ DM)] / 100$$

Endogenous protein loss in the small intestine (ENDP) is associated with undigested dry matter (UDM).

$$UDM\ (g/kg\ DM) = (ash \times 0.35) + [OM - (OM \times dOM / 100)]$$

$$ENDP\ (g/kg\ DM) = 0.075 \times UDM\ (g/kg\ DM)$$

Where, 0.35 indicates 35% of ash is assumed not digested, dOM is digestibility of organic matter in the in situ 48 h rumen incubation, and 0.075 indicates 75 g protein is absorbed per kilogram of UDM to compensate for endogenous loss.

The difference between potential microbial protein synthesis based on rumen degradable protein and potential protein synthesis based on energy provided by rumen fermentation was estimated as degraded protein balance (OEB).

$$OEB\ (g/kg\ DM) = RDP\ (g/kg\ DM) - MCP_{FOM}\ (g/kg\ DM)$$

3.2.8.2. NRC Dairy Model

Metabolizable protein, similar to DVE, is considered as truly absorbed rumen-undegraded feed protein in the small intestine (ARUP), truly absorbed microbial protein in the small intestine (AMCP) and truly absorbed rumen endogenous protein in the small intestine (AECP) (NRC Dairy, 2001).

$$MP\ (g/kg\ DM) = AMCP^{NRC}\ (g/kg\ DM) + ARUP^{NRC}\ (g/kg\ DM) + AECP^{NRC}\ (g/kg\ DM)$$

Unlike the DVE/OEB system, the NRC Dairy model is based on TDN, and endogenous protein is considered part of total digested protein in the small intestine. The estimation of microbial protein synthesis related to TDN at a production level ($TDN_{3\times}$), with an efficiency about 0.13.

$$MCP_{TDN}\ (g/kg\ DM) = 0.13 \times TDN_{3\times}\ (g/kg\ DM)$$

If rumen degraded protein (EDCP from the in situ rumen degradation trials) exceeds 1.18 times MCP_{TDN}, then MCP_{TDN} would be used as MCP^{NRC}. Oppositely, MCP^{NRC} would be calculated as:

$$MCP^{NRC}\ (g/kg\ DM) = 0.85 \times RDP^{NRC}$$

Where, 0.85 indicates the approximate efficiency of RDP converting to microbial protein.

Truly absorbed microbial protein in the small intestine ($AMCP^{NRC}$) was then calculated as:

$$AMCP^{NRC}\ (g/kg\ DM) = 0.80 \times 0.80 \times MCP^{NRC}$$

Where, 0.80 represents approximately 80% of microbial crude protein existing as amino acids, of which 80% is digested in the small intestine.

The estimation of rumen undegraded feed protein ($ARUP^{NRC}$) is based on in situ rumen degradation results.

$$ARUP^{NRC}\ (g/kg\ DM) = [\%dBCP\ (\%) \times RUP^{NRC}\ (g/kg\ DM)] / 100$$

With rumen endogenous crude protein (ECP) considered part of intestinal digestion, the truly absorbed rumen endogenous protein in the small intestine (AECP) was then calculated as:

$$ECP\ (g/kg\ DM) = 6.25 \times 1.9 \times DM\ (kg)$$

$$AECP\ (g/kg\ DM) = 0.50 \times 0.80 \times ECP\ (g/kg\ DM)$$

Where, 6.25 is the conversion factor of N to protein, 1.9 represents 1.9 g endogenous N produced per 1kg DM, 0.50 and 0.80 indicate that 80% endogenous crude protein is true protein, of which only 50% passes to the small intestine.

The concept of degraded protein balance (DPB) is obtained from the DVE/OEB system (Yu, 2005c) as DPB (g/kg DM) = EDCP (g/kg DM) – 1.18 MCP_{TDN} (g/kg DM).

3.2.8.3. Predicted Feed Milk Value

Truly digested protein absorbed by the small intestine is used in part to produce milk. The feed milk value (FMV) is used to predict the efficiency of feed true protein for milk production (Theodoridou and Yu, 2013b). The efficiency of metabolizable protein (MP) for lactation is assumed to be 0.67, and 1 kg milk contains approximate 33 g milk crude protein (NRC Dairy, 2001; Theodoridou and Yu, 2013b). Therefore, FMV can be estimated as below:

$$FMV\ (kg\ milk/kg\ DM) = 0.67 \times MP\ (g/kg\ DM) / 33$$

Where, MP is the metabolizable protein (DVE value in DVE/OEB system).

3.2.9. Statistical Analyses

The statistical analyses of chemical composition, energy values, protein and carbohydrate fractions, rumen degradation of nutrients, hourly effective degradation ratios, intestinal digestion of protein, predicted truly absorbed protein supply and feed milk values were performed using the MIXED procedure of SAS 9.3 (SAS Institute, Inc., Cary, NC, US). The model used for the

analyses of chemical and anti-nutrient profiles, energy values, protein and carbohydrate fractions is $Y_{ij} = \mu + F_i + e_{ij}$, where Y_{ij} is an observation of the dependent variable ij, μ is the population mean for the variable, F_i is the effect of different feeds as a fixed effect (different sources are treated as replication), and e_{ij} is the random error associated with the observation ij. For in situ rumen degradation kinetics, hourly effective degradation ratios, in vitro intestinal digestion of protein and truly absorbed protein supply predictions, the model used for analyses is $Y_{ij} = \mu + F_i + S_j + e_{ij}$, where Y_{ij} is an observation of the dependent variable ij, μ is the population mean for the variable, F_i is the effect of different feeds as a fixed effect, S_j is the run effect in the in situ trial as a random effect, and e_{ij} is the random error associated with the observation ij. Contrast statements were used in the oilseed study to compare the differences between new carinata seeds and new canola seeds; new carinata seeds and commercial canola seeds; yellow seeds and brown seeds. For all statistical analyses, significance was declared at $P < 0.05$, and trends at $0.05 \leq P \leq 0.10$.

3.3. Results and Discussion

3.3.1. Chemical Profiles and Anti-nutritional Compounds

3.3.1.1. Chemical Profiles and Anti-nutritional Compounds of Carinata Seeds in Comparison with Canola Seeds

As shown in Table 3.2.1, according to contrast results, new carinata seeds had significantly higher DM compared with both new canola seeds and the commercial canola seed ($P<0.05$), but all canola seeds were shown to have higher average crude fat than carinata seeds. The yellow carinata seeds was similar to the brown carinata seed in DM, ash and EE. The CP and NPN were higher in the yellow carinata seed (30.5 %DM and 58.6 %SCP respectively), significantly greater than those of canola seeds. However, the yellow carinata seed was lower in NDICP than the brown carinata seed and canola seeds ($P<0.05$). There was no significant difference between the brown

carinata seed and the brown canola seed in protein profiles. Simbaya et al. (1995) indicated yellow-seeded *Brassica* meal contained lower fiber content than brown-seeded meals. In our study, significant differences were found between yellow and brown seeds in NDF, ADF and ADL based on contrast P-values ($P<0.05$). New yellow carinata seed had the lowest NDF compared with other seeds.

New carinata seeds were much higher in total glucosinolates (93.9 μmol/g for the yellow carinata seed and 90.1 μmol/g for the brown carinata seed), most of which was allyl glucosinolate (Table 3.2.2). This high content of glucosinolates in *Brassica carinata* would reduce palatability and thus intake by animals, and may cause potential growth or health problems (especially for monogastric and young animals), which would be an obstacle to further utilization in animal feed industry (Hill, 1991; Bell, 1993; Tripathi and Mishra, 2007). Condensed tannins in carinata seeds and canola seeds were not significantly different in our study. The yellow carinata seed had similar glucosinolates and condensed tannins to the brown carinata seed.

3.3.1.2. Chemical Profiles and Anti-nutritional Compounds of Carinata Meal and Hexane-extracted Carinata Presscake in Comparison with Canola Meal

Table 3.3.1 shows the chemical composition of carinata meal, hexane-extracted carinata presscake and the commercial canola meal. There was no significant difference between carinata meal and canola meal for ash content, however carinata meal (92.7%) had higher DM than canola meal (90.2%). Carinata meal showed similar CP, NPN and NDICP compared with canola meal, but had twice the SCP. Fiber content (NDF, ADF and ADL) was lower in carinata meal than canola meal ($P<0.05$). Canola presscake had similar fiber content, CP, SCP and NPN compared with canola meal, however, the DM, OM and EE in canola presscake were higher (Theodoridou and Yu, 2013c). In our study, with hexane-extraction, more of the oil portion was removed in carinata presscake than carinata meal (0.3 %DM vs. 2.5 %DM, $P<0.05$). However, hexane-extracted

carinata presscake appeared to have higher SCP (72.6 %CP) than carinata meal (53.0 %CP) and canola meal (24.0 %CP), but similar DM, ash, CP and fiber contents to carinata meal. The increase of SCP may be due to the cold-pressing, which caused the rupture of primary bonds holding the chains of amino acids together, and made crude protein more soluble. The findings by McKinnon and Walker (2009) showed similar effects of cold pressing on canola seed.

Thacker and Petri (2009) found canola presscake had higher total glucosinolates than canola meal (12.67 μmol/g vs. 8.78 μmol/g). Cold pressing with hexane extraction did not significantly affect total glucosinolates in carinata presscake (168.5 μmol/g vs. 115.2 μmol/g in carinata meal, $P>0.05$), of which most was allyl glucosinolate (Table 3.3.2). Several treatments could be conducted to reduce glucosinolates in carinata co-products before their utilization in animal diets. Huang et al. (1995) found extrusion with heat successfully reduced glucosinolate content of high-glucosinolate (116 μmol/g) rapeseed meal, especially in low moisture conditions. However, Jensen et al. (1995) found cold extrusion after cold pressing may not influence anti-nutritional compounds without the use of heat before or during extrusion. Condensed tannins were not significantly different among two carinata co-products and canola meal ($P>0.05$).

3.3.2. Energy Values

3.3.2.1. Energy Values of Carinata Seeds in Comparison with Canola Seeds

As shown in Table 3.4, new carinata seeds were significantly different from new canola seeds and the commercial canola seed in total digestible nutrients ($TDN_{1\times}$) and energy values according to contrast P-values ($P<0.05$). Yellow carinata seed had higher tdCP but similar energy values when compared with the yellow canola seed, while the brown carinata seed was lower in $TDN_{1\times}$ and energy content than the new brown canola seed. Given the hull color effect, yellow seeds contained more average energy than brown seeds ($P<0.05$). Moreover, yellow carinata seed

had similar $NE_{Lp3\times}$ to both new canola seeds and the commercial variety, which may provide equivalent net energy for lactation.

3.3.2.2. Energy Values of Carinata Meal and Hexane-extracted Carinata Presscake in Comparison with Canola Meal

No significant difference was found among three co-products in tdNFC, tdNDF and tdFA ($P>0.05$), but tdCP was higher in two carinata co-products (meal and presscake), as Table 3.5 shows. These carinata co-products were higher in $TDN_{1\times}$ compared to canola meal, while the carinata meal was similar to canola meal in energy values. Theodoridou and Yu (2013c) found brown canola presscake had higher energy values and similar $TDN_{1\times}$ compared to brown canola meal. Hexane-extracted carinata presscake did not show significantly different total digestible nutrients and energy content in this study, but had higher digestible energy, metabolizable energy and net energy suited to dairy cows at a production level (three times maintenance) than canola meal. In summary, even with less oil, hexane-extracted carinata presscake was similar to carinata meal in energy values but higher than commercial canola meal, which may indicate that it could be considered a superior feed energy source for ruminants.

Table 3.2.1 Chemical profiles of new carinata seeds (Yellow-AAC A110 vs. Brown-110915EM) in comparison with new canola seeds (Yellow-YN07 C1386 vs. Brown-N07 1374) and a commercial canola seed (Brown)

Components	New Carinata Seeds (N_CN)		New Canola Seeds (N_CL)		Commercial Canola Seed (COMM)	SEM	P value	Contrast, P value		
	Yellow (AAC A110)	Brown (110915EM)	Yellow (YN07 C1386)	Brown (N07 1374)	Brown			N_CN vs N_CL	Yellow vs Brown	N_CN vs. COMM
DM (%)	96.5^{a}	96.1^{ab}	95.5^{ab}	94.7^{b}	95.0^{ab}	0.27	0.03	0.01	0.08	0.01
Ash (%DM)	4.1^{ab}	4.3^{a}	3.6^{b}	3.7^{b}	4.0^{ab}	0.11	0.02	0.003	0.18	0.11
EE (%DM)	42.4^{bc}	38.6^{c}	48.2^{a}	45.4^{ab}	47.7^{a}	0.92	0.003	0.001	0.02	0.001
Protein profile										
CP (%DM)	30.5^{a}	26.0^{ab}	23.2^{b}	23.8^{b}	22.8^{b}	0.95	0.01	0.004	0.09	0.01
SCP (%CP)	63.9	52.6	58.6	60.7	65.3	2.44	0.07	0.59	0.12	0.07
NPN (%SCP)	58.6^{a}	37.6^{b}	18.2^{c}	20.3^{bc}	19.1^{bc}	3.37	0.001	<0.001	0.04	0.001
NDICP (%CP)	1.5^{b}	4.6^{a}	4.7^{a}	6.2^{a}	5.5^{a}	0.32	0.001	0.001	0.001	0.001
ADICP (%CP)	0.6^{b}	2.5^{a}	1.8^{ab}	3.5^{a}	3.5^{a}	0.32	0.01	0.02	0.002	0.004
Carbohydrate profile										
NDF (%DM)	6.2^{c}	12.4^{a}	9.2^{b}	13.0^{a}	12.1^{a}	0.25	<0.001	0.001	<0.001	<0.001
ADF (%DM)	3.6^{d}	7.1^{bc}	5.0^{cd}	9.6^{a}	8.8^{ab}	0.43	0.001	0.01	<0.001	0.001
ADL (%DM)	0.2^{c}	2.3^{bc}	0.9^{c}	4.9^{a}	4.3^{ab}	0.39	0.001	0.01	0.001	0.002
NFC (%DM)	17.3	19.9	17.0	15.5	14.7	0.94	0.06	0.05	0.59	0.02

Notes: SEM: standard error of the mean. DM: dry matter; EE: ether extracts (crude fat); CP: crude protein; SCP: soluble crude protein; NPN: non-protein nitrogen; NDICP: neutral detergent insoluble crude protein; ADICP: acid detergent insoluble crude protein; NDF: neutral detergent fiber; ADF: acid detergent fiber; ADL: acid detergent lignin; NFC: non-fiber carbohydrate. Means with different superscripts in the same row are significantly different according to Tukey method (P<0.05).

Table 3.2.2 Glucosinolates and condensed tannins in new carinata seeds (Yellow-AAC A110 vs. Brown-110915EM) in comparison with new canola seeds (Yellow-YN07 C1386 vs. Brown-N07 1374) and a commercial canola seed (Brown)

Components	New Carinata Seeds (N_CN)		New Canola Seeds (N_CL)		Commercial Canola Seed (COMM)	SEM	P value	Contrast, P value		
	Yellow (AAC A110)	Brown (110915 EM)	Yellow (YN07 C1386)	Brown (N07 1374)	Brown			N_CN vs N_CL	Yellow vs Brown	N_CN vs. COMM
Glucosinolates (μmol/g)										
allyl glucosinolate	87.3[a]	83.8[a]	0.1[b]	0.0[b]	0.0[b]	4.50	<0.001	<0.001	0.71	<0.001
3-butenyl glucosinolate	1.0	1.2	1.3	0.7	1.8	0.32	0.31	0.92	0.57	0.12
4-pentenyl glucosinolate	0.03	0.04	0.13	0.14	0.27	0.062	0.17	0.18	0.92	0.03
2-OH-3-butenyl glucosinolate	1.3	2.0	1.5	1.9	4.8	0.80	0.12	0.87	0.52	0.02
2-OH-4-pentenyl glucosinolate	0.06	0.06	0.00	0.03	0.24	0.084	0.40	0.63	0.89	0.14
CH_3-thiobutenyl glucosinolate	0.00[b]	0.00[b]	0.11[ab]	0.07[ab]	0.15[a]	0.027	0.01	0.01	0.38	0.002
phenylethyl glucosinolate	0.51[a]	0.20[ab]	0.12[b]	0.04[b]	0.12[b]	0.060	0.02	0.01	0.02	0.02
CH_3-thiopentenyl glucosinolate	0.00[b]	0.00[b]	0.08[a]	0.03[ab]	0.08[a]	0.011	0.01	0.01	0.06	0.003
4-OH-benzyl glucosinolate	0.1[a]	0.2[a]	0.0[b]	0.0[b]	0.0[b]	0.01	0.002	<0.001	0.10	<0.001
3-CH_3-indolyl glucosinolate	0.4[a]	0.1[b]	0.4[a]	0.2[b]	0.4[a]	0.03	0.002	0.09	<0.001	0.004
4-OH-3-CH_3-indolyl glucosinolate	3.4	2.7	4.0	3.4	4.6	0.34	0.07	0.09	0.12	0.01
total glucosinolates	93.9[a]	90.1[a]	7.8[b]	6.7[b]	12.4[b]	4.97	<0.001	<0.001	0.64	<0.001
Condensed tannins (abs/mg/ml)	0.027	0.030	0.023	0.027	0.027	0.002	0.37	0.14	0.20	0.61

Notes: "-": not detectable. Abs: absorbance. SEM: standard error of the mean. Means with different superscripts in the same row are significantly different according to Tukey method (P<0.05).

Table 3.3.1 Chemical profiles of carinata meal and hexane-extracted carinata presscake in comparison with canola meal

Components	Carinata Meal	Carinata Presscake	Canola Meal	SEM	P value
DM (%)	92.7[a]	93.9[a]	90.2[b]	0.32	0.003
Ash (%DM)	7.1	6.8	7.1	0.37	0.80
EE (%DM)	2.5[a]	0.3[b]	1.8[ab]	0.42	0.04
Protein profile					
CP (%DM)	48.5[ab]	53.5[a]	39.7[b]	2.50	0.04
SCP (%CP)	53.0[b]	72.6[a]	24.0[c]	3.49	0.001
NPN (%SCP)	67.9[a]	38.9[b]	82.4[a]	3.89	0.003
NDICP (%CP)	6.0[ab]	2.9[b]	10.6[a]	1.10	0.02
ADICP (%CP)	1.6[b]	0.6[b]	3.8[a]	0.37	0.01
Carbohydrate profile					
NDF (%DM)	14.5[b]	10.2[b]	25.8[a]	1.97	0.01
ADF (%DM)	10.2[b]	6.7[b]	19.4[a]	1.09	0.003
ADL (%DM)	2.7[b]	0.9[b]	9.0[a]	0.51	0.001
NFC (%DM)	30.2	30.7	29.9	1.04	0.86

Notes: SEM: standard error of the mean. DM: dry matter; EE: ether extracts (crude fat); CP: crude protein; SCP: soluble crude protein; NPN: non-protein nitrogen; NDICP: neutral detergent insoluble crude protein; ADICP: acid detergent insoluble crude protein; NDF: neutral detergent fiber; ADF: acid detergent fiber; ADL: acid detergent lignin; NFC: non-fiber carbohydrate. Means with different superscripts in the same row are significantly different according to Tukey method ($P<0.05$).

Table 3.3.2 Glucosinolates and condensed tannins in carinata meal and hexane-extracted carinata presscake in comparison with canola meal

Components	Carinata Meal	Carinata Presscake	Canola Meal	SEM	P value
Glucosinolates (μmol/g)					
allyl glucosinolate	109.9[a]	159.3[a]	0.0[b]	16.22	0.01
3-butenyl glucosinolate	0.8	1.2	0.7	0.16	0.16
4-pentenyl glucosinolate	0.11[a]	0.12[a]	0.05[b]	0.007	0.004
2-OH-3-butenyl glucosinolate	1.8	1.9	1.9	0.34	0.95
2-OH-4-pentenyl glucosinolate	0.06	0.10	0.06	0.011	0.06
CH_3-thiobutenyl glucosinolate	0.03	0.03	0.06	0.018	0.57
phenylethyl glucosinolate	0.2[b]	0.5[a]	0.0[c]	0.03	<0.001
CH3-thiopentenyl glucosinolate	0.00	0.02	0.00	0.014	0.60
4-OH-benzyl glucosinolate	0.10[ab]	0.20[a]	0.07[b]	0.024	0.03
3-CH_3-indolyl glucosinolate	0.3	0.5	0.1	0.09	0.07
4-OH-3-CH_3-indolyl glucosinolate	1.9[b]	4.5[a]	0.5[b]	0.53	0.01
total glucosinolates	115.2[a]	168.5[a]	3.4[b]	16.93	0.01
Condensed tannins (abs/mg/ml)	0.049	0.043	0.034	0.0033	0.08

Notes: "-": not detectable. Abs: absorbance. SEM: standard error of the mean. Means with different superscripts in the same row are significantly different according to Tukey method ($P<0.05$).

Table 3.4 Energy values of new carinata seeds (Yellow-AAC A110 vs. Brown-110915EM) in comparison with new canola seeds (Yellow-YN07 C1386 vs. Brown-N07 1374) and a commercial canola seed (Brown)

Components	New Carinata Seeds (N_CN)		New Canola Seeds (N_CL)		Commercial Canola Seed (COMM)	SEM	P value	Contrast, P value		
	Yellow (AAC A110)	Brown (110915EM)	Yellow (YN07 C1386)	Brown (N07 1374)	Brown			N_CN vs N_CL	Yellow vs Brown	N_CN vs. COMM
Total digestible nutrient (%DM)										
tdNFC	16.9	19.5	16.6	15.2	14.4	0.92	0.06	0.05	0.59	0.02
tdCP	30.5^{a}	25.7^{ab}	23.0^{b}	23.5^{b}	22.5^{b}	0.93	0.01	0.004	0.07	0.005
tdNDF	3.6^{a}	4.3^{a}	4.2^{a}	2.2^{b}	2.3^{b}	0.24	0.003	0.02	0.04	0.002
tdFA	41.4^{bc}	37.6^{c}	47.2^{a}	44.4^{ab}	46.7^{a}	0.92	0.003	0.001	0.02	0.001
Total digestible nutrients (%DM)										
$TDN_{1\times}$	137.1^{b}	127.2^{c}	143.0^{a}	133.8^{b}	137.3^{b}	0.98	0.001	0.001	<0.001	0.01
Energy value (Mcal/kg)										
$DE_{p3\times}$, dairy	5.66^{ab}	5.22^{d}	5.79^{a}	5.44^{c}	5.56^{bc}	0.031	<0.001	0.002	<0.001	0.03
$ME_{p3\times}$, dairy	5.45^{ab}	4.98^{d}	5.60^{a}	5.24^{c}	5.37^{bc}	0.035	<0.001	0.002	<0.001	0.01
$NE_{Lp3\times}$, dairy	3.93^{ab}	3.56^{c}	4.09^{a}	3.80^{b}	3.92^{ab}	0.032	0.001	0.002	<0.001	0.01
ME, beef	5.05^{ab}	4.66^{d}	5.17^{a}	4.86^{c}	4.97^{bc}	0.027	<0.001	0.002	<0.001	0.02
NE_{m}, beef	3.63^{ab}	3.33^{d}	3.73^{a}	3.48^{c}	3.57^{bc}	0.019	<0.001	0.001	<0.001	0.01
NE_{g}, beef	2.66^{ab}	2.42^{d}	2.73^{a}	2.54^{c}	2.61^{bc}	0.015	<0.001	0.001	<0.001	0.01

Notes: SEM: standard error of the mean. tdNFC: truly digestible non-fiber carbohydrate; tdCP: total digestible crude protein; tdNDF: total digestible neutral detergent fiber; tdFA: total digestible fatty acid; $TDN_{1\times}$: total digestible nutrients; $DE_{p3\times}$: digestible energy at a production level (3× maintenance); $ME_{p3\times}$: metabolizable energy at a production level (3× maintenance); $NE_{Lp3\times}$: Net energy at a production level (3× maintenance); ME: metabolizable energy; NE_{m}: net energy for maintenance; NE_{g}: net energy for gain. Means with different superscripts in the same row are significantly different according to Tukey method (P<0.05).

Table 3.5 Energy values of carinata meal and hexane-extracted carinata presscake in comparison with canola meal

Components	Carinata Meal	Carinata Presscake	Canola Meal	SEM	P value
Truly digestible nutrient (%DM)					
tdNFC	29.6	30.1	29.3	1.02	0.86
tdCP	48.2^{ab}	53.4^{a}	39.0^{b}	2.54	0.04
tdNDF	4.3	4.5	4.2	0.59	0.88
tdFA	1.5	0.0	0.8	0.42	0.13
Total digestible nutrients (%DM)					
$TDN_{1\times}$	78.5^{a}	81.0^{a}	67.3^{b}	2.02	0.02
Energy value (Mcal/kg)					
$DE_{p3\times}$, dairy	3.64^{ab}	3.80^{a}	3.09^{b}	0.110	0.02
$ME_{p3\times}$, dairy	3.23^{ab}	3.39^{a}	2.68^{b}	0.110	0.02
$NE_{Lp3\times}$, dairy	2.08^{ab}	2.19^{a}	1.69^{b}	0.077	0.02
ME, beef	3.25^{ab}	3.40^{a}	2.76^{b}	0.096	0.02
NE_m, beef	2.24^{ab}	2.35^{a}	1.83^{b}	0.079	0.02
NE_g, beef	1.55^{ab}	1.65^{a}	1.20^{b}	0.065	0.02

Notes: SEM: standard error of the mean. tdNFC: truly digestible non-fiber carbohydrate; tdCP: total digestible crude protein; tdNDF: total digestible neutral detergent fiber; tdFA: total digestible fatty acid; $TDN_{1\times}$: total digestible nutrients; $DE_{p3\times}$: digestible energy at a production level (3× maintenance); $ME_{p3\times}$: metabolizable energy at a production level (3× maintenance); $NE_{Lp3\times}$: Net energy at a production level (3× maintenance); ME: metabolizable energy; NE_m: net energy for maintenance; NE_g: net energy for gain. Means with different superscripts in the same row are significantly different according to Tukey method ($P<0.05$).

3.3.3. Protein and Carbohydrate Fractions

3.3.3.1. Protein and Carbohydrate Fractions of Carinata Seeds in Comparison with Canola Seeds

According to the CNCPS, protein fractions were similar among carinata seeds and the new canola seeds ($P>0.05$), except for the PC fraction (Table 3.6.1). Compared with the commercial canola seed, carinata seeds had similar soluble true protein (PA2) and slowly-degradable true protein (PB2) ($P>0.05$) but significantly higher moderately-degradable true protein (PB1) ($P<0.05$). Seeds with brown hulls exhibited more undegradable protein (PC) than yellow seeds, while no significant difference existed between carinata seeds and canola seeds with the same seed coat color. Brown carinata seed had more total CHO and less sugar (CA4) than the yellow line, while no significant differences were observed in other carbohydrate fractions between them. New carinata seeds were different from new canola seeds and the commercial canola seed in CB2, CB3 and CC in accordance with contrast P-values ($P<0.05$). Yellow seeds showed much lower amount of unavailable NDF (CC fraction) than brown seeds (carinata: 2.5 %CHO vs. 18.0 %CHO; canola: 8.4 %CHO vs. 43.4 %CHO, respectively), which resulted from their thinner hulls (Chungu et al., 2015).

Based on the degradation and passage rates in the CNCPS library (NDS Software, Ruminant Management & Nutrition, Reggio Emilia, Italy), as shown in Table 3.6.2, yellow carinata seed had the most rumen-degraded protein (RDP: 22.2 %DM), while other seeds contained similar RDP. It was also found that yellow carinata seed had significantly less total rumen undegraded CHO than the brown carinata seed (6.4 %DM vs. 12.3%). In conclusion, carinata seeds had relatively higher average total ruminally degraded and bypass protein (TRDP and TRUP), as well as a higher average total ruminally degraded CHO (TRDC), whereas their average total ruminally undegraded CHO (TRUC) was lower (contrast $P<0.05$).

3.3.3.2. Protein and Carbohydrate Fractions of Carinata Meal and Hexane-extracted Carinata Presscake in Comparison with Canola Meal

Protein fractions of the three co-products are shown in Table 3.7.1, with carinata meal lower in PB1 and PC than canola meal. However, carinata meal had more soluble true protein than canola meal (53.0 %CP and 24.0 %CP, respectively). There was no obvious difference found in PB2 fraction between carinata meal and canola meal ($P>0.05$). For carbohydrate partitions, carinata meal had greater CB3 but less CC than canola meal, while other CHO fractions were similar ($P>0.05$). The cold pressing affected protein solubility (72.6 %CP) and moderately degradable protein fraction (24.5 %CP), with no influence on CHO fractions. To summarize, the two carinata co-products had more PA2 and CB3 but less PB1, PC and CC than canola meal, which means less rumen undegradable protein and CHO in carinata co-products.

In terms of rumen degradation characteristics (Table 3.7.2), carinata meal had significantly lower total rumen undegraded CHO compared to canola meal ($P<0.05$), with similar degraded or bypass protein, based on the degradation and passage rates obtained in the CNCPS library (NDS Software, Ruminant Management & Nutrition, Reggio Emilia, Italy). Hexane-extracted carinata presscake had higher total RDP and lower TRUC than the commercial canola meal ($P<0.05$), however, it was not significantly different from carinata meal in terms of total rumen degraded or bypass protein and CHO.

Table 3.6.1 Protein and carbohydrate fractions of new carinata seeds (Yellow-AAC A110 vs. Brown-110915EM) in comparison with new canola seeds (Yellow-YN07 C1386 vs. Brown-N07 1374) and a commercial canola seed (Brown) based on CNCPS

Components	New Carinata Seeds (N_CN)		New Canola Seeds (N_CL)		Commercial Canola Seed (COMM)	SEM	P value	Contrast, P value		
	Yellow (AAC A110)	Brown (110915EM)	Yellow (YN07 C1386)	Brown (N07 1374)	Brown			N_CN vs N_CL	Yellow vs Brown	N_CN vs. COMM
Protein fractions										
PA2 (%CP)	63.9	52.6	58.6	60.7	65.3	2.44	0.07	0.59	0.12	0.07
PB1 (%CP)	34.6	42.8	36.7	33.1	29.2	2.35	0.06	0.16	0.38	0.02
PB2 (%CP)	1.0	2.0	2.9	2.7	2.0	0.52	0.22	0.06	0.45	0.44
PC (%CP)	0.6^{b}	2.5^{a}	1.8^{ab}	3.5^{a}	3.5^{a}	0.32	0.01	0.02	0.002	0.004
Carbohydrate fractions										
CHO (%DM)	23.0^{b}	31.1^{a}	25.0^{b}	27.0^{ab}	25.5^{b}	0.72	0.004	0.22	0.001	0.13
CA4 (%CHO)	25.8^{a}	19.7^{b}	27.8^{a}	21.3^{b}	20.5^{b}	0.73	0.002	0.06	<0.001	0.05
CB1 (%CHO)	0.8	1.4	1.6	0.9	1.0	0.31	0.38	0.71	0.89	0.72
CB2 (%CHO)	48.6^{a}	42.8^{ab}	38.4^{ab}	35.0^{b}	36.0^{ab}	2.23	0.03	0.01	0.09	0.02
CB3 (%CHO)	20.3^{a}	17.7^{ab}	24.4^{a}	0.1^{c}	5.1^{bc}	2.45	0.004	0.04	0.003	0.01
CC (%CHO)	2.5^{b}	18.0^{ab}	8.4^{b}	43.4^{a}	40.7^{a}	4.51	0.004	0.02	0.003	0.003

Notes: SEM: standard error of the mean. PA2: rapidly degradable true protein (soluble true protein); PB1: moderately degradable true protein. PB2: slowly degradable true protein (bound in NDF); PC: undegradable protein; CHO: carbohydrate; CA4: sugar; CB1: starch; CB2: soluble fiber; CB3: available NDF; CC: unavailable NDF; Means with different superscripts in the same row are significantly different according to Tukey method ($P<0.05$).

Table 3.6.2 Predicted rumen degraded and undegraded fractions of protein and carbohydrate in new carinata seeds (Yellow-AAC A110 vs. Brown-110915EM) in comparison with new canola seeds (Yellow-YN07 C1386 vs. Brown-N07 1374) and a commercial canola seed (Brown) based on CNCPS

Components	New Carinata Seeds (N_CN)		New Canola Seeds (N_CL)		Commercial Canola Seed (COMM)	SEM	P value	Contrast, P value		
	Yellow (AAC A110)	Brown (110915EM)	Yellow (YN07 C1386)	Brown (N07 1374)	Brown			N_CN vs N_CL	Yellow vs Brown	N_CN vs. COMM
Rumen degraded protein fractions										
RDPA2 (%DM)	15.0^{a}	10.5^{b}	10.5^{b}	11.1^{b}	11.5^{ab}	0.63	0.02	0.03	0.03	0.15
RDPB1 (%DM)	7.1^{a}	7.4^{a}	5.7^{ab}	5.3^{ab}	4.5^{b}	0.43	0.02	0.01	0.97	0.003
RDPB2 (%DM)	0.1	0.2	0.3	0.3	0.2	0.05	0.30	0.09	0.45	0.70
TRDP (%DM)	22.2^{a}	18.2^{b}	16.4^{b}	16.7^{b}	16.1^{b}	0.68	0.01	0.003	0.04	0.005
Rumen undegraded protein fractions										
RUPA2 (%DM)	4.5^{a}	3.2^{b}	3.1^{b}	3.3^{b}	3.4^{ab}	0.19	0.02	0.03	0.03	0.15
RUPB1 (%DM)	3.5^{a}	3.7^{a}	2.8^{ab}	2.6^{ab}	2.2^{b}	0.22	0.02	0.01	0.97	0.004
RUPB2 (%DM)	0.2	0.3	0.4	0.4	0.3	0.06	0.27	0.08	0.41	0.67
RUPC (%DM)	0.2^{b}	0.7^{a}	0.4^{ab}	0.8^{a}	0.8^{a}	0.08	0.01	0.04	0.003	0.01
TRUP (%DM)	8.4^{a}	7.8^{ab}	6.8^{b}	7.2^{ab}	6.7^{b}	0.27	0.03	0.01	0.78	0.01
Rumen degraded carbohydrate fractions										
RDCA4 (%DM)	4.6	4.7	5.3	4.4	4.0	0.22	0.06	0.30	0.14	0.07
RDCB1 (%DM)	0.2	0.4	0.3	0.2	0.2	0.07	0.31	0.95	0.60	0.56
RDCB2 (%DM)	9.3	11.1	8.0	7.9	7.7	0.66	0.06	0.02	0.27	0.03
RDCB3 (%DM)	2.1^{ab}	2.5^{a}	2.8^{a}	0.01^{c}	0.6^{bc}	0.29	0.003	0.02	0.01	0.005
TRDC (%DM)	16.1^{ab}	18.7^{a}	16.5^{ab}	12.5^{b}	12.3^{b}	1.06	0.03	0.04	0.52	0.01
Rumen undegraded carbohydrate fractions										
RUCA4 (%DM)	1.4	1.4	1.6	1.3	1.2	0.07	0.06	0.32	0.15	0.07
RUCB1 (%DM)	0.04	0.09	0.08	0.05	0.05	0.018	0.28	0.89	0.52	0.46
RUCB2 (%DM)	1.9	2.2	1.6	1.6	1.5	0.13	0.06	0.02	0.27	0.03

Table 3.6.2 Cont'd

Components	New Carinata Seeds (N_CN)		New Canola Seeds (N_CL)		Commercial Canola Seed (COMM)	SEM	P value	Contrast, P value		
	Yellow (AAC A110)	Brown (110915EM)	Yellow (YN07 C1386)	Brown (N07 1374)	Brown			N_CN vs N_CL	Yellow vs Brown	N_CN vs. COMM
RUCB3 (%DM)	2.6^{ab}	3.0^{a}	3.3^{a}	0.01^{c}	0.7^{bc}	0.34	0.003	0.02	0.01	0.005
RUCC (%DM)	0.6^{c}	5.6^{bc}	2.1^{c}	11.7^{a}	10.3^{ab}	0.95	0.001	0.01	0.001	0.002
TRUC (%DM)	6.4^{b}	12.3^{a}	8.7^{b}	14.6^{a}	13.5^{a}	0.45	<0.001	0.004	<0.001	0.001

Notes: SEM: standard error of the mean. RDPA2: ruminally degraded PA2; RDPB1: ruminally degraded PB1; RDPB2: ruminally degraded PB2; TRDP: total ruminally degraded CP; RUPA2: ruminally undegraded PA2; RUPB1: ruminally undegraded PB1; RUPB2: ruminally undegraded PB2; RUPC: ruminally undegraded PC; TRUP: total ruminally undegraded CP; RDCA4: ruminally degraded CA4; RDCB1: ruminally degraded CB1; RDCB2: ruminally degraded CB2; RDCB3: ruminally degraded CB3; TRDC: total ruminally degraded carbohydrate; RUCA4: ruminally undegraded CA4; RUCB1: ruminally undegraded CB1; RUCB2: ruminally undegraded CB2; RUCB3: ruminally undegraded CB3; RUCC: ruminally undegraded CC; TRUC: total ruminally undegraded carbohydrate. Means with different superscripts in the same row are significantly different according to Tukey method ($P<0.05$).

Table 3.7.1 Protein and carbohydrate fractions of carinata meal and hexane-extracted carinata presscake in comparison with canola meal based on CNCPS

Components	Carinata Meal	Carinata Presscake	Canola Meal	SEM	P value
Protein fractions					
PA2 (%CP)	53.0[b]	72.6[a]	24.0[c]	3.49	0.001
PB1 (%CP)	41.0[b]	24.5[c]	65.4[a]	2.53	0.001
PB2 (%CP)	4.4	2.3	6.8	0.91	0.06
PC (%CP)	1.6[b]	0.6[b]	3.8[a]	0.37	0.01
Carbohydrate fractions					
CHO (%DM)	41.9	39.4	51.5	2.63	0.06
CA4 (%CHO)	26.1	32.1	21.7	3.19	0.16
CB1 (%CHO)	2.2	1.2	1.0	0.64	0.46
CB2 (%CHO)	44.1	44.7	35.4	4.56	0.38
CB3 (%CHO)	17.9[a]	18.6[a]	3.1[b]	0.99	0.001
CC (%CHO)	14.9[b]	5.6[b]	42.1[a]	2.13	0.001

Notes: SEM: standard error of the mean. PA2: rapidly degradable true protein (soluble true protein); PB1: moderately degradable true protein. PB2: slowly degradable true protein (bound in NDF); PC: undegradable protein; CHO: carbohydrate; CA4: sugar; CB1: starch; CB2: soluble fiber; CB3: available NDF; CC: unavailable NDF; Means with different superscripts in the same row are significantly different according to Tukey method ($P<0.05$).

Table 3.7.2 Predicted rumen degraded and undegraded fractions of protein and carbohydrate in carinata meal and hexane-extracted carinata presscake in comparison with canola meal based on CNCPS

Components	Carinata Meal	Carinata Presscake	Canola Meal	SEM	P value
Rumen degraded protein fractions					
RDPA2 (%DM)	20.0^{ab}	29.9^{a}	7.3^{b}	2.44	0.01
RDPB1 (%DM)	13.8^{b}	9.1^{c}	18.2^{a}	0.52	0.001
RDPB2 (%DM)	0.8	0.5	1.1	0.14	0.09
TRDP (%DM)	34.6^{ab}	39.6^{a}	26.6^{b}	2.12	0.03
Rumen undegraded protein fractions					
RUPA2 (%DM)	6.0^{ab}	9.0^{a}	2.2^{b}	0.73	0.01
RUPB1 (%DM)	5.9^{b}	3.9^{c}	7.8^{a}	0.23	0.001
RUPB2 (%DM)	1.2	0.7	1.6	0.21	0.08
RUPC (%DM)	0.7^{ab}	0.3^{b}	1.5^{a}	0.16	0.01
TRUP (%DM)	13.9	14.0	13.1	0.38	0.33
Rumen degraded carbohydrate fractions					
RDCA4 (%DM)	8.4	9.8	8.6	1.19	0.67
RDCB1 (%DM)	0.8	0.4	0.5	0.27	0.52
RDCB2 (%DM)	15.3	14.6	15.2	1.15	0.89
RDCB3 (%DM)	3.0^{a}	2.9^{a}	0.6^{b}	0.27	0.01
TRDC (%DM)	27.5	27.7	24.9	1.07	0.24
Rumen undegraded carbohydrate fractions					
RUCA4 (%DM)	2.5	2.9	2.6	0.36	0.66
RUCB1 (%DM)	0.15	0.07	0.08	0.046	0.54
RUCB2 (%DM)	3.1	2.9	3.0	0.23	0.89
RUCB3 (%DM)	4.1^{a}	4.0^{a}	0.9^{b}	0.37	0.01
RUCC (%DM)	6.5^{b}	2.2^{b}	21.7^{a}	1.23	0.001
TRUC (%DM)	16.3^{b}	12.2^{b}	28.2^{a}	1.64	0.01

Table 3.7.2 Cont'd

Notes: SEM: standard error of the mean. RDPA2: ruminally degraded PA2; RDPB1: ruminally degraded PB1; RDPB2: ruminally degraded PB2; TRDP: total ruminally degraded CP; RUPA2: ruminally undegraded PA2; RUPB1: ruminally undegraded PB1; RUPB2: ruminally undegraded PB2; RUPC: ruminally undegraded PC; TRUP: total ruminally undegraded CP; RDCA4: ruminally degraded CA4; RDCB1: ruminally degraded CB1; RDCB2: ruminally degraded CB2; RDCB3: ruminally degraded CB3; TRDC: total ruminally degraded carbohydrate; RUCA4: ruminally undegraded CA4; RUCB1: ruminally undegraded CB1; RUCB2: ruminally undegraded CB2; RUCB3: ruminally undegraded CB3; RUCC: ruminally undegraded CC; TRUC: total ruminally undegraded carbohydrate. Means with different superscripts in the same row are significantly different according to Tukey method ($P<0.05$).

3.3.4. Rumen Degradation Kinetics of Nutrients

3.3.4.1. Rumen Degradation Kinetics of Carinata Seeds in Comparison with Canola Seeds

The rumen degradation kinetics of organic matter (OM) are outlined in Table 3.8.1. No significant differences were observed in Kd and T0 of OM among all oil seeds, while new carinata seeds were different from new canola seeds in soluble and degradable fractions ($P<0.05$). Amongst all, yellow carinata seed had the highest S fraction (25.1%), but yellow canola seed had the greatest D fraction (82.8%). However, brown carinata seed had similar rumen OM degradation features to the new brown canola seed. Yellow seeds of carinata and canola had the least U fractions ($P<0.05$), which were close to zero. Moreover, yellow carinata seed had the highest EDOM (651 g/kg DM) amongst all seeds, which may provide more available energy for N utilization in the rumen and milk protein synthesis (Shabi et al., 1998).

The CP degradation rates of new carinata seeds and new canola seeds were similar, though new carinata seeds had significantly different Kd values compared with the commercial canola seed ($P<0.05$) (Table 3.8.2). Carinata seeds and canola seeds were similar in lag time (T0) ($P>0.05$). Yellow carinata seed had a higher S fraction (29.4%) and a lower U fraction (1.0%) among all seed lines, while brown carinata seed had similar S, D and U fractions to the newly developed brown canola seed. Based on the DVE/OEB system and the NRC Dairy model, carinata seeds were high in both ruminal degraded and bypass crude protein (EDCP and RUP), with the yellow seed having higher EDCP (232 g/kg DM). The brown carinata seed had higher EDCP than brown canola seeds, but similar RUP. Santos et al. (1998) pointed out that a high RUP diet usually decreased microbial protein synthesis, but a high RUP and RDP source of supplemental protein would increase milk yield. Given this, carinata seed may be considered as an alternative good protein source with high RUP and EDCP. According to the RDP prediction based on CNCPS ststem, the in situ rumen degradation results were similar to the prediction of rumen CP degradation

characteristics, which proved the accuracy of using the CNCPS model to predict protein degradation kinetics of feedstuffs.

Table 3.8.3 shows the NDF degradability features of the five oil seeds. Carinata seeds had significantly different T0 values compared with new canola seeds ($P<0.05$), but were similar to commercial canola seed ($P>0.05$). NDF in canola seeds was degraded more rapidly in the rumen compared to carinata seeds. The commercial canola seed had the highest degradation rate (Kd), following by the new brown canola seed. However, no significant difference of D and U fractions was found between carinata and canola seeds of the same hull color. Yellow lines had more D fraction and less U fraction than brown lines, but brown lines had significantly higher rumen bypass NDF ($P<0.05$). New canola seeds had the most EDNDF, and yellow carinata seed had the least.

3.3.4.2. Rumen Degradation Kinetics of Carinata Meal and Hexane-extracted Carinata Presscake in Comparison with Canola Meal

As the results show in Table 3.9.1, carinata meal had similar Kd, T0 and degradable (D) fractions to canola meal, but a higher S fraction and lower U fraction. This reflected the higher EDOM of carinata meal versus canola meal. McKinnon and Walker (2009) found pressing improved S fraction of DM in canola presscake but reduced D and U fractions of DM. The cold pressing treatment in our study changed OM degradability in the rumen, by increasing Kd, T0 and soluble fraction but reducing D and U fractions. This processing significantly enhanced EDOM, which may supply more energy for microbial activities in the rumen (Shabi et al., 1998).

Carinata meal had similar Kd, T0 and U fraction of crude protein to canola meal (Table 3.9.2), while carinata meal contained more S fraction and less D fraction of CP ($P<0.05$). The rumen undegraded CP was lower in carinata meal (115 g/kg based on NRC Dairy), but carinata meal was greater in EDCP (370 g/kg DM) than canola meal (235 g/kg DM). As McKinnon and

Walker (2009) have shown, canola cold presscake had more soluble CP fraction but lower D fraction of CP than traditional canola meal. A higher EDCP was observed in canola presscake relative to canola meal. Our results exhibited enhancements of Kd, T0 and S fraction of CP and reduction of D fraction through cold pressing of carinata seeds. The hexane-extracted carinata presscake had lower RUP but higher EDCP compared to conventionally processed carinata meal ($P<0.05$). However, based on prediction results according to the CNCPS system, no significant difference in rumen CP degradability was found between carinata meal and canola meal, as for hexane-extracted carinata presscake. Theodoridou and Yu (2013c) studied the rumen degradation features of canola meal and canola presscake, and found no significant change of EDCP and RUCP by processing, except an improvement of degradation rate (Kd) of crude protein.

No significant differences were observed in Kd and T0 of NDF among the three co-products ($P>0.05$), except carinata meal and canola meal had smaller degradable fractions than hexane-extracted carinata presscake (65.7%, 47.6% vs. 90.5%, respectively). McKinnon and Walker (2009) reported that cold pressing of canola would decrease the undegradable fraction of NDF and raise EDNDF. However, a different study indicated that presscake processing may not change rumen degradability of NDF (Theodoridou and Yu, 2013c). Among the three co-products, canola meal had the highest EDNDF (73 g/kg DM), followed by carinata meal and hexane-extracted carinata presscake (48 g/kg DM). No effect of cold pressing was found on the EDNDF value, though hexane-extracted carinata presscake had lower RUNDF than carinata meal (54 vs. 98 g/kg DM).

Table 3.8.1 In situ rumen degradation kinetics of organic matter (OM) in new carinata seeds (Yellow-AAC A110 vs. Brown-110915EM) in comparison with new canola seeds (Yellow-YN07 C1386 vs. Brown-N07 1374) and a commercial canola seed (Brown)

Components	New Carinata Seeds (N_CN)		New Canola Seeds (N_CL)		Commercial Canola Seed (COMM)	SEM	P value	Contrast, P value		
	Yellow (AAC A110)	Brown (110915 EM)	Yellow (YN07 C1386)	Brown (N07 1374)	Brown			N_CN vs N_CL	Yellow vs Brown	N_CN vs. COMM
In situ rumen OM degradation										
Kd (%/h)	7.98	7.73	7.53	9.24	9.29	0.579	0.13	0.38	0.23	0.06
T0 (h)	0.22	0.03	0.07	0.37	0.30	0.141	0.41	0.52	0.71	0.33
S (%)	25.1^{a}	18.9^{bc}	17.0^{c}	18.0^{bc}	20.8^{b}	0.86	<0.001	<0.001	0.01	0.27
D (%)	74.9^{b}	75.4^{b}	82.8^{a}	74.4^{b}	69.5^{c}	0.97	<0.001	0.003	0.001	<0.001
U (%)	0.0^{c}	5.7^{b}	0.2^{c}	7.7^{ab}	9.7^{a}	0.57	<0.001	0.07	<0.001	<0.001
%BOM (%RUOM)	32.2^{b}	38.8^{a}	36.9^{a}	37.4^{a}	37.1^{a}	0.89	0.001	0.08	0.001	0.17
BOM (RUOM, g/kg DM)	309^{b}	371^{a}	356^{a}	360^{a}	356^{a}	8.5	0.001	0.049	0.001	0.14
%EDOM (%RDOM)	67.8^{a}	61.2^{b}	63.1^{b}	62.6^{b}	62.9^{b}	0.89	0.001	0.08	0.001	0.17
EDOM (RDOM, g/kg DM)	651^{a}	586^{b}	608^{b}	603^{b}	604^{b}	8.7	0.001	0.17	0.001	0.22

Notes: SEM: standard error of the mean. Kd: the degradation rate of D fraction; T0: lag time; S: soluble fraction in the in situ incubation; D: degradable fraction; U: rumen undegradable fraction; BOM or RUOM: rumen bypass organic matter; EDOM or RDOM: effective degraded organic matter; Kp: passage rate. Means with different superscripts in the same row are significantly different according to Tukey method ($P<0.05$).

Table 3.8.2 In situ rumen degradation kinetics of crude protein (CP) in new carinata seeds (Yellow-AAC A110 vs. Brown-110915EM) in comparison with new canola seeds (Yellow-YN07 C1386 vs. Brown-N07 1374) and a commercial canola seed (Brown)

Components	New Carinata Seeds (N_CN)		New Canola Seeds (N_CL)		Commercial Canola Seed (COMM)	SEM	P value	Contrast, P value		
	Yellow (AAC A110)	Brown (110915 EM)	Yellow (YN07 C1386)	Brown (N07 1374)	Brown			N_CN vs N_CL	Yellow vs Brown	N_CN vs. COMM
In situ rumen CP degradation										
Kd (%/h)	12.19^{b}	14.39^{ab}	14.35^{ab}	13.23^{ab}	15.22^{a}	0.637	0.04	0.45	0.41	0.03
T0 (h)	0.00	0.02	0.00	0.06	0.11	0.050	0.55	0.73	0.44	0.15
S (%)	29.4^{a}	23.9^{bc}	18.7^{c}	22.1^{bc}	24.6^{ab}	1.21	<0.001	<0.001	0.40	0.19
D (%)	69.6^{ab}	70.4^{ab}	75.6^{a}	71.2^{ab}	67.4^{b}	1.39	0.01	0.03	0.21	0.15
U (%)	1.0^{c}	5.7^{b}	5.7^{b}	6.7^{ab}	8.0^{a}	0.53	<0.001	<0.001	<0.001	<0.001
%BCP (%CP)	24.0^{c}	26.5^{b}	28.1^{ab}	29.0^{a}	27.1^{ab}	0.53	<0.001	<0.001	0.01	0.01
RUP^{NRC} (g/kg DM)	73^{a}	69^{ab}	65^{ab}	69^{ab}	62^{b}	2.0	0.01	0.06	0.91	0.002
BCP^{DVE} (g/kg DM)	81^{a}	77^{ab}	72^{ab}	77^{ab}	69^{b}	2.3	0.01	0.06	0.91	0.002
%EDCP (%RDP, %CP)	76.0^{a}	73.5^{b}	71.9^{bc}	71.0^{c}	72.9^{bc}	0.53	<0.001	<0.001	0.01	0.01
EDCP (RDP, g/kg DM)	232^{a}	191^{b}	167^{c}	169^{c}	167^{c}	4.1	<0.001	<0.001	<0.001	<0.001

Notes: SEM: standard error of the mean. Kd: the degradation rate of D fraction; T0: lag time; S: soluble fraction in the in situ incubation; D: degradable fraction; U: rumen undegradable fraction; BCP^{DVE}: rumen bypass or undegraded feed crude protein in DVE/OEB model; RUP^{NRC}: rumen bypass or undegraded feed crude protein in NRC Dairy 2001 model; EDCP or RDP: effective degraded feed crude protein; Kp: passage rate. Means with different superscripts in the same row are significantly different according to Tukey method (P<0.05).

Table 3.8.3 In situ rumen degradation kinetics of neutral detergent fiber (NDF) in new carinata seeds (Yellow-AAC A110 vs. Brown-110915EM) in comparison with new canola seeds (Yellow-YN07 C1386 vs. Brown-N07 1374) and a commercial canola seed (Brown)

Components	New Carinata Seeds (N_CN)		New Canola Seeds (N_CL)		Commercial Canola Seed (COMM)	SEM	P value	Contrast, P value		
	Yellow (AAC A110)	Brown (110915 EM)	Yellow (YN07 C1386)	Brown (N07 1374)	Brown			N_CN vs N_CL	Yellow vs Brown	N_CN vs. COMM
In situ rumen NDF degradation										
Kd (%/h)	5.81^{c}	8.48^{c}	11.04^{c}	19.53^{b}	26.49^{a}	1.202	<0.001	<0.001	<0.001	<0.001
T0 (h)	0.10	0.16	0.95	0.23	0.35	0.266	0.06	0.04	0.12	0.39
D (%)	100.0^{a}	60.5^{b}	89.2^{a}	58.8^{b}	44.2^{c}	3.07	<0.001	0.06	<0.001	<0.001
U (%)	0.0^{c}	39.5^{b}	10.8^{c}	41.2^{b}	55.8^{a}	3.07	<0.001	0.06	<0.001	<0.001
%BNDF (%RUNDF)	51.1^{cd}	65.0^{a}	42.8^{d}	55.1^{bc}	64.0^{ab}	2.26	<0.001	0.001	<0.001	0.049
BNDF (RUNDF, g/kg DM)	32^{b}	80^{a}	39^{b}	72^{a}	77^{a}	2.6	<0.001	0.84	<0.001	<0.001
%EDNDF (%RDNDF)	48.9^{ab}	35.0^{d}	57.2^{a}	44.9^{bc}	36.0^{cd}	2.26	<0.001	0.001	<0.001	0.049
EDNDF (RDNDF, g/kg DM)	30^{c}	43^{b}	52^{ab}	59^{a}	43^{b}	2.4	<0.001	<0.001	0.001	0.03

Notes: SEM: standard error of the mean. Kd: the degradation rate of D fraction; T0: lag time; D: degradable fraction; U: rumen undegradable fraction; BDNDF or RUNDF: rumen bypass or undegraded feed neutral detergent fiber; EDNDF or RDNDF: effective degraded neutral detergent fiber; Kp: passage rate. Means with different superscripts in the same row are significantly different according to Tukey method (P<0.05).

Table 3.9.1 In situ rumen degradation kinetics of organic matter (OM) in carinata meal and hexane-extracted carinata presscake in comparison with canola meal

Components	Carinata Meal	Carinata Presscake	Canola Meal	SEM	P value
In situ rumen OM degradation					
Kd (%/h)	15.49^{b}	23.93^{a}	9.62^{b}	2.537	0.001
T0 (h)	0.00^{b}	1.17^{a}	0.00^{b}	0.134	<0.001
S (%)	15.1^{b}	44.0^{a}	9.2^{c}	0.78	<0.001
D (%)	71.3^{a}	49.2^{b}	71.0^{a}	1.38	<0.001
U (%)	13.6^{b}	6.8^{c}	19.8^{a}	1.43	<0.001
%BOM (%RUOM)	33.5^{b}	17.0^{c}	47.2^{a}	1.16	<0.001
BOM (RUOM, g/kg DM)	312^{b}	158^{c}	439^{a}	10.4	<0.001
%EDOM (%RDOM)	66.5^{b}	83.0^{a}	52.8^{c}	1.16	<0.001
EDOM (RDOM, g/kg DM)	618^{b}	774^{a}	490^{c}	11.7	<0.001

Notes: SEM: standard error of the mean. Kd: the degradation rate of D fraction; T0: lag time; S: soluble fraction in the in situ incubation; D: degradable fraction; U: rumen undegradable fraction; BOM or RUOM: rumen bypass organic matter; EDOM or RDOM: effective degraded organic matter; Kp: passage rate. Means with different superscripts in the same row are significantly different according to Tukey method (P<0.05).

Table 3.9.2 In situ rumen degradation kinetics of crude protein (CP) in carinata meal and hexane-extracted carinata presscake in comparison with canola meal

Components	Carinata Meal	Carinata Presscake	Canola Meal	SEM	P value
In situ rumen CP degradation					
Kd (%/h)	22.09^{b}	37.79^{a}	10.35^{b}	4.425	0.002
T0 (h)	0.00^{b}	1.07^{a}	0.00^{b}	0.202	0.003
S (%)	14.0^{b}	45.1^{a}	2.8^{c}	1.29	<0.001
D (%)	78.7^{b}	51.9^{c}	89.5^{a}	1.00	<0.001
U (%)	7.2^{ab}	3.0^{b}	7.7^{a}	1.14	0.02
%BCP (%CP)	24.1^{b}	10.7^{c}	40.8^{a}	1.56	<0.001
RUP^{NRC} (g/kg DM)	115^{b}	57^{c}	162^{a}	5.2	<0.001
BCP^{DVE} (g/kg DM)	128^{b}	64^{c}	180^{a}	5.8	<0.001
%EDCP (%RDP, %CP)	75.9^{b}	89.3^{a}	59.2^{c}	1.56	<0.001
EDCP (RDP, g/kg DM)	370^{b}	478^{a}	235^{c}	18.2	<0.001

Notes: SEM: standard error of the mean. Kd: the degradation rate of D fraction; T0: lag time; S: soluble fraction in the in situ incubation; D: degradable fraction; U: rumen undegradable fraction; BCP^{DVE}: rumen bypass or undegraded feed crude protein in DVE/OEB model; RUP^{NRC}: rumen bypass or undegraded feed crude protein in NRC Dairy 2001 model; EDCP or RDP: effective degraded feed crude protein; Kp: passage rate. Means with different superscripts in the same row are significantly different according to Tukey method ($P<0.05$).

Table 3.9.3 In situ rumen degradation kinetics of neutral detergent fiber (NDF) in carinata meal and hexane-extracted carinata presscake in comparison with canola meal

Components	Carinata Meal	Carinata Presscake	Canola Meal	SEM	P value
In situ rumen NDF degradation					
Kd (%/h)	6.65	6.93	8.81	0.801	0.18
T0 (h)	1.77	2.23	3.37	0.480	0.11
D (%)	65.7^{b}	90.5^{a}	47.6^{b}	5.71	0.001
U (%)	34.3^{a}	9.5^{b}	52.4^{a}	5.71	0.001
%BNDF (%RUNDF)	66.4^{a}	53.0^{b}	71.8^{a}	1.95	<0.001
BNDF (RUNDF, g/kg DM)	98^{b}	54^{c}	185^{a}	9.0	<0.001
%EDNDF (%RDNDF)	33.6^{b}	47.1^{a}	28.2^{b}	1.95	<0.001
EDNDF (RDNDF, g/kg DM)	48^{b}	48^{b}	73^{a}	4.1	0.002

Notes: SEM: standard error of the mean. Kd: the degradation rate of D fraction; T0: lag time; D: degradable fraction; U: rumen undegradable fraction; BDNDF or RUNDF: rumen bypass or undegraded feed neutral detergent fiber; EDNDF or RDNDF: effective degraded neutral detergent fiber; Kp: passage rate. Means with different superscripts in the same row are significantly different according to Tukey method ($P<0.05$).

3.3.5. Hourly Effective Rumen Degradation Ratios

3.3.5.1. Hourly Effective Rumen Degradation Ratios of Carinata Seeds in Comparison with Canola Seeds

The effective rumen degradation ratios of N to OM (ED_N/ED_OM) were 57 g N/kg OM and 52 g N/kg OM for the new yellow and brown carinata seeds, relatively higher than canola seeds (Table 3.10). There was no significant difference observed between yellow and brown seeds for the ratios of ED_N to ED_OM. The optimal ratio between effective degradation of ED_N and ED_OM (energy) is 25 g N/kg OM (Tamminga et al., 1990; Sinclair et al., 1993), with minimum potential N loss and maximum microbial synthesis. For ratios above the optimal 25 g N/kg OM, the feed may contain redundant N or deficient energy, which potentially result in inadequate utilization of nitrogen. Conversely, there would be nitrogen shortage for microbial activities. Therefore, the higher ratios of carinata seeds indicates that carinata seeds may have more potential N loss than canola seeds, in accordance with their dietary CP contents and energy values.

The hourly effective degradation ratios amongst all seeds are shown in Figure 3.1. There was a rapid increase of ED_N to ED_OM ratios for all seed samples during 0 h to 2 h incubation. At 0 h, carinata seeds had higher ED_N/ED_OM ratios than the canola seeds ($P<0.05$), without hull color effect. The ED_N to ED_OM ratios dropped from 2 h for all the seeds, but only the yellow carinata seed remained higher than the optimal rumen fermentation ratio at 24 h (27 g N/kg OM). The other seeds had ratios under the optimal N to energy ratio at 24 h, which would result in N shortages for rumen fermentation when given as the sole feed source. This may result from the higher effective degradable crude protein of yellow carinata seed than other seeds. Hull color did not significantly influence hourly effective degradation ratios of N to OM throughout the degradation process ($P>0.05$).

3.3.5.2. Hourly Effective Rumen Degradation Ratios of Carinata Meal and Hexane-extracted Carinata Presscake in Comparison with Canola Meal

The effective degradation ratio of N to OM was significantly higher in carinata meal (95 g N/kg OM) than in canola meal (77 g N/kg OM) ($P<0.05$), which is also higher than the optimal N to energy ratio (25 g N/kg OM) (Table 3.11). This may indicate extra N in the rumen for microbial synthesis (Nuez-Ortín and Yu, 2010). The hexane-extracted carinata presscake did not show a significantly different ED_N/ED_OM ratio compared with carinata meal in our study (99 g N/kg OM) ($P>0.05$).

The hourly effective degradation ratio of N to OM of carinata meal was higher than that of canola meal (78 g/kg vs. 21 g/kg) at the beginning (0 h). Subsequently, dramatic increases of ED_N to ED_OM ratios were observed between 0 h to 2 h for three co-products, with the ratio of canola meal improving the most (Figure 3.2). A gradual increase of ED_N to ED_OM ratios was observed after 2 h rumen incubation for canola meal in another study of canola meal (Huang et al., 2015a). In this study, the ED_N/ED_OM ratios of canola meal decreased slowly from 92 g/kg at 2 h to 78 g/kg at 24 h, however the ED_N/ED_OM ratios of carinata meal dropped faster from 119 g/kg at 2 h to 29 g/kg at 24 h. At 24 h, the ratio for carinata meal was close to optimal, which may suggest that this co-product as a superior source of RDP and energy. Initially, hexane-extracted carinata presscake had a similar ED_N/ED_OM ratio with carinata meal, but the ratios decreased faster and finally reached 14 g N/kg OM at 24 h (<25 g N/kg OM), which may potentially result in a N shortage for dairy cattle. The cold pressing in this study improved degradation rates of OM and CP, which may explain why ED_N/ED_OM ratios of hexane-extracted carinata presscake decreased faster than carinata meal.

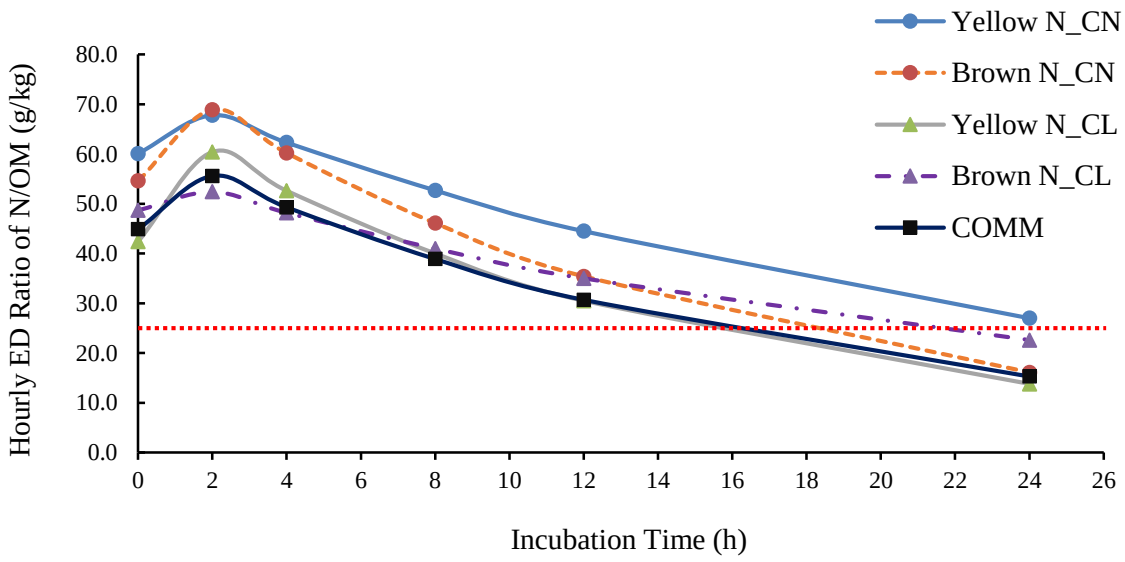


Figure 3.1 Hourly effective degradation ratios between ED_N and ED_OM of new carinata seeds (Yellow-AAC A110 vs. Brown-110915EM) in comparison with new canola seeds (Yellow-YN07 C1386 vs. Brown-N07 1374) and a commercial canola seed (Brown) (optimal ED_N to ED_OM ratio is 25g N/kg OM)

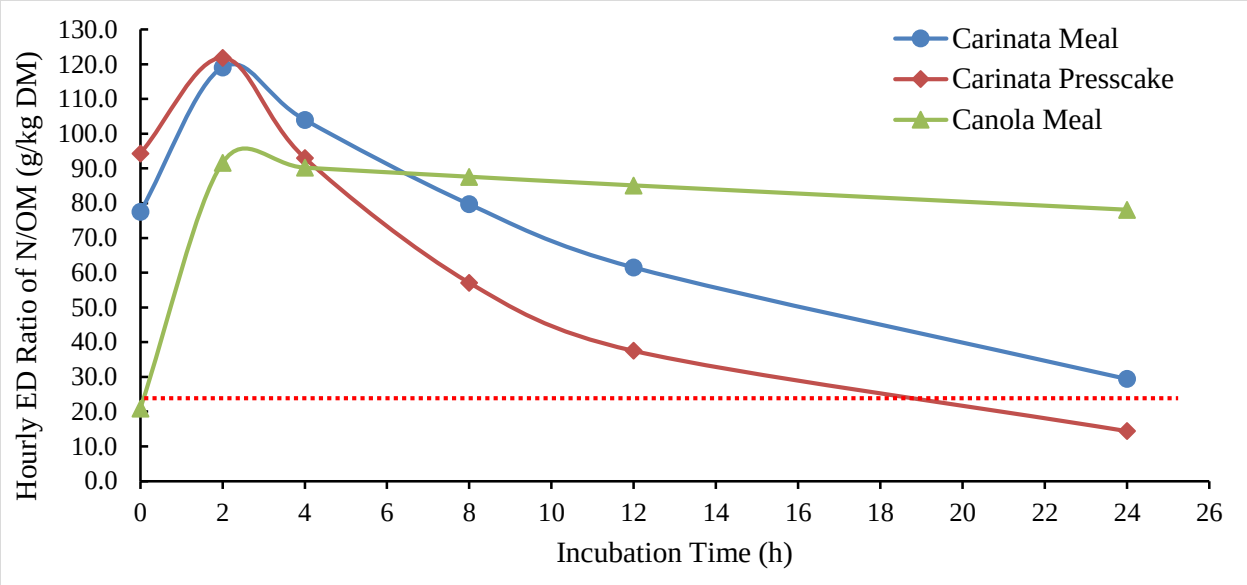


Figure 3.2 Hourly effective degradation ratios between ED_N and ED_OM of carinata meal and hexane-extracted carinata presscake in comparison with canola meal (optimal ED_N to ED_OM ratio is 25g N/kg OM)

Table 3.10 Rumen degradation ratios between available nitrogen (ED_N) and available organic matter (ED_OM) and hourly effective rumen degradation ratios of new carinata seeds (Yellow-AAC A110 vs. Brown-110915EM) in comparison with new canola seeds (Yellow-YN07 C1386 vs. Brown-N07 1374) and a commercial canola seed (Brown)

Components	New Carinata Seeds (N_CN)		New Canola Seeds (N_CL)		Commercial Canola Seed (COMM)	SEM	P value	Contrast, P value		
	Yellow (AAC A110)	Brown (110915EM)	Yellow (YN07 C1386)	Brown (N07 1374)	Brown			N_CN vs N_CL	Yellow vs Brown	N_CN vs. COMM
N to OM ratio (g/kg)										
N / OM	51^{a}	43^{b}	38^{c}	40^{bc}	38^{c}	0.9	<0.001	<0.001	0.003	<0.001
ED_N / ED_OM	57^{a}	52^{a}	44^{b}	45^{b}	44^{b}	1.2	<0.001	<0.001	0.15	<0.001
Hourly effective degradation ratios of N to OM at individual times (g/kg)										
h0	60^{a}	55^{ab}	42^{c}	49^{bc}	45^{c}	1.8	<0.001	<0.001	0.81	<0.001
h2	68^{a}	69^{a}	60^{ab}	52^{b}	56^{ab}	3.4	0.01	0.003	0.32	0.01
h4	62^{a}	60^{a}	53^{ab}	48^{b}	49^{b}	2.5	0.003	0.001	0.21	0.001
h8	53^{a}	46^{ab}	40^{bc}	41^{bc}	39^{c}	1.5	<0.001	<0.001	0.09	<0.001
h12	45^{a}	35^{b}	31^{b}	35^{b}	31^{b}	1.6	<0.001	<0.001	0.16	<0.001
h24	27^{a}	16^{b}	14^{b}	23^{ab}	15^{b}	2.3	0.005	0.16	0.66	0.04

Notes: SEM: standard error of the mean; ED_N: effective degraded nitrogen; ED_OM: effective degraded organic matter. Means with different superscripts in the same row are significantly different according to Tukey method (P<0.05).

Table 3.11 Rumen degradation ratios between available nitrogen (ED_N) and available organic matter (ED_OM) and hourly effective rumen degradation ratios of carinata meal and hexane-extracted carinata presscake in comparison with canola meal

Components	Carinata Meal	Carinata Presscake	Canola Meal	SEM	P value
N to OM ratio (g/kg)					
N / OM	83[a]	92[a]	68[b]	2.4	<0.001
ED_N / ED_OM	95[a]	99[a]	77[b]	3.0	0.001
Hourly effective degradation ratios of N to OM at individual times (g/kg)					
h0	78[a]	94[a]	21[b]	6.9	<0.001
h2	119[a]	122[a]	92[b]	4.3	0.001
h4	104	93	90	4.9	0.16
h8	80[ab]	57[b]	88[a]	7.7	0.03
h12	62[ab]	38[b]	85[a]	8.5	0.01
h24	29[b]	14[b]	78[a]	6.5	<0.001

Notes: SEM: standard error of the mean; ED_N: effective degraded nitrogen; ED_OM: effective degraded organic matter. Means with different superscripts in the same row are significantly different according to Tukey method (P<0.05).

3.3.6. Intestinal Digestion of Protein

3.3.6.1. Intestinal Protein Digestion of Carinata Seeds in Comparison with Canola Seeds

Intestinal digestibility of RUP (dIDP) showed no significant difference between new carinata seeds and new canola seeds (P>0.05), but carinata seeds had higher intestinal protein digestibility (average 44.1 %) and IDP (average 31 g/kg DM) than the commercial canola seed (34.6%, 21 g/kg DM) (Table 3.12). Hull color had no significant influence on intestinal protein digestibility in this study, however Theodoridou and Yu (2013c) discovered that yellow-seeded canola meal had higher IDP than brown-seeded canola meal. Khan et al. (2014) also found yellow flaxseeds had significantly higher IDCP than brown flaxseeds (P<0.001).

In our study, yellow carinata seed had the highest total digested CP (259 g/kg DM), followed by brown carinata seed (226 g/kg DM) (P<0.05). The two carinata seeds showed significantly greater total protein digestibility than canola seeds (contrast P<0.05), which may be a consequence of the higher rumen degraded CP content.

3.3.6.2. Intestinal Protein Digestion of Carinata Meal and Hexane-extracted Carinata Presscake in Comparison with Canola Meal

Canola meal showed a superior advantage in providing more intestinal digested crude protein (97 g/kg DM) compared to carinata meal (71 g/kg DM), as shown in Table 3.13. This might be a result of less amount of RUP passing from the rumen provided by carinata meal. Similarly, Theodoridou and Yu (2013c) observed that the brown-hull canola presscake had less IDP than brown-hull canola. However, with higher RDP, carinata meal showed obviously higher total digested feed protein (TDP) than canola meal (441 g/kg DM vs. 332 g/kg DM). The cold pressing in this study increased the Kd and soluble fraction of CP in the rumen incubation, which led to less RUP but more RDP, and consequently resulted in less intestinal digested CP for hexane-extracted

carinata presscake compared with carinata meal ($P<0.05$). However, the hexane-extracted carinata presscake had a higher total digested feed crude protein (504 g/kg DM) than carinata meal ($P<0.05$).

Table 3.12 Intestinal digested and total digested crude protein in new carinata seeds (Yellow-AAC A110 vs. Brown-110915EM) in comparison with new canola seeds (Yellow-YN07 C1386 vs. Brown-N07 1374) and a commercial canola seed (Brown)

Components	New Carinata Seeds (N_CN)		New Canola Seeds (N_CL)		Commercial Canola Seed (COMM)	SEM	P value	Contrast, P value		
	Yellow (AAC A110)	Brown (110915EM)	Yellow (YN07 C1386)	Brown (N07 1374)	Brown			N_CN vs N_CL	Yellow vs Brown	N_CN vs. COMM
Intestinal crude protein digestion										
dIDP (% RUP)	37.3^{b}	50.8^{a}	50.4^{a}	36.7^{b}	34.6^{b}	2.96	0.002	0.88	0.98	0.02
IDP (% CP)	8.9^{c}	13.5^{ab}	14.2^{a}	10.7^{bc}	9.3^{c}	0.80	0.001	0.15	0.53	0.07
IDP (g/kg DM)	27^{ab}	35^{a}	33^{a}	25^{ab}	21^{b}	2.3	0.01	0.36	0.95	0.003
Total crude protein digestion										
TDP (% CP)	84.9^{ab}	87.0^{a}	86.1^{a}	81.7^{b}	82.2^{b}	0.87	0.002	0.03	0.20	0.004
TDP (g/kg DM)	259^{a}	226^{b}	199^{c}	195^{c}	188^{c}	5.7	<0.001	<0.001	0.004	<0.001

Notes: SEM: standard error of the mean. dIDP: intestinal digestibility of rumen bypass protein on percentage basis; IDP: intestinal digested crude protein; TDP: total digested crude protein. Means with different superscripts in the same row are significantly different according to Tukey method ($P<0.05$).

Table 3.13 Intestinal digested and total digested crude protein in carinata meal and hexane-extracted carinata presscake in comparison with canola meal

Components	Carinata Meal	Carinata Presscake	Canola Meal	SEM	P value
Intestinal crude protein digestion					
dIDP (% RUP)	62.2[a]	45.7[b]	59.9[a]	2.91	0.003
IDP (% CP)	14.9[b]	4.9[c]	24.5[a]	1.04	<0.001
IDP (g/kg DM)	71[b]	26[c]	97[a]	3.3	<0.001
Total crude protein digestion					
TDP (% CP)	90.8[b]	94.1[a]	83.7[c]	0.83	<0.001
TDP (g/kg DM)	441[b]	504[a]	332[c]	16.1	<0.001

Notes: SEM: standard error of the mean. dIDP: intestinal digestibility of rumen bypass protein on percentage basis; IDP: intestinal digested crude protein; TDP: total digested crude protein. Means with different superscripts in the same row are significantly different according to Tukey method (P<0.05).

3.3.7. Predicted Truly Absorbed Protein Supply to Dairy Cattle and Feed Milk Value

3.3.7.1. Predicted Truly Absorbed Protein Supply and Feed Milk Value of Carinata Seeds in Comparison with Canola Seeds

The DVE/OEB system was used to predict truly absorbed protein supply of carinata seeds for dairy cattle (Table 3.14.1). Carinata seeds had higher truly absorbed microbial protein in the small intestine (average $AMCP^{DVE}$: 40 g/kg DM) than both new and commercial canola seeds (contrast $P<0.05$), given the higher MCP synthesized in the rumen based on available N. Among all seeds, carinata seeds had similar truly absorbed bypass protein in the small intestine compared to new canola seeds, but a higher $ABCP^{DVE}$ value than the commercial canola seed ($P<0.05$). Therefore, the two carinata seeds were significantly higher in truly digested protein in the small intestine (DVE: 68 g/kg DM and 69 g/kg DM for yellow and brown cultivars) compared with brown canola seeds, however, a similar DVE was observed for new yellow canola seed (67 g/kg DM). The higher truly digested protein contributed to higher feed milk values of new carinata seeds and the new yellow canola seed (1.4 kg milk/kg feed) based on the DVE/OEB system. According to Tamminga et al. (1994; 2007), a positive degraded protein balance (OEB) in a diet indicated potential N loss for dairy cows. Xin et al. (2013c) found similar OEB values among carinata seeds and canola seeds, and hull color had no significant effect on OEB values of carinata seeds. Our results differed in that carinata seeds had higher OEB and yellow carinata seed had a greater balance than the brown cultivar.

According to Table 3.14.2, the protein supply of carinata seeds was predicted based on the NRC Dairy model (NRC Dairy, 2001), showing dairy cows would have a higher true protein value if fed new carinata seeds. Among all the seeds, yellow and brown carinata seeds had significantly higher metabolizable protein (MP: 137 g/kg DM) in the small intestine, including microbial protein, rumen-bypass protein and endogenous protein. The new yellow canola seed competitively had

similar MP with carinata seeds (128 g/kg DM). According to findings based on the DVE/OEB system, no significant difference was observed in ARUP for all seeds ($P>0.05$). Therefore, carinata seeds were higher in feed milk values (2.8 kg milk/kg feed), followed by the brown canola seed (2.5 kg milk/kg feed). Like the OEB results based on DVE/OEB system, DPB values of carinata seeds were greater than those of canola seeds, with the yellow seed relatively higher than the brown ($P<0.05$). However, unlike in the DVE/OEB system, canola seeds had negative degraded protein balance values in the NRC Dairy model, which indicated potential N shortage. In addition, comparing yellow seeds with brown seeds, hull color had a significant effect on AECP in the NRC Dairy model ($P<0.01$).

3.3.7.2. Predicted Truly Absorbed Protein Supply and Feed Milk Value of Carinata Meal and Hexane-extracted Carinata Presscake in Comparison with Canola Meal

Carinata meal had more truly absorbed rumen synthesized microbial protein (AMCP), but less absorbed bypass protein (ABCP) in the small intestine compared with canola meal based on the DVE/OEB system ($P<0.05$) (Table 3.15.1), which eventually contributed to similar DVE and feed milk values between them (133 g/kg DM vs. 143 g/kg DM; 2.7 kg milk/kg feed vs. 2.9 kg milk/kg feed, respectively). For degraded protein balance, carinata meal was higher than canola meal (257 g/kg DM vs. 136 g/kg DM), which may result in potential N loss. The hexane-extracted carinata presscake, which had high AMCP (78 g/kg DM) and low ABCP (29 g/kg DM), supplied significantly less truly digested protein in the small intestine of dairy cattle (DVE: 101 g/kg DM), but had a higher OEB value (350 g/kg DM) compared to carinata meal ($P<0.05$). This may result from the increase of protein degradability (solubility) in the rumen during the pressing. Nonetheless, Theodoridou and Yu (2013b) found no significant difference between brown-seeded canola presscake and brown-seeded canola meal on ABCP and OEB values. Cold pressing for carinata in our study impaired rumen bypass protein and total protein that truly digested in the

small intestine of dairy cattle. In conclusion, based on the DVE/OEB system, carinata meal may be considered a similar protein source to canola meal considering its competitive protein supply to the small intestine of dairy cattle, whereas the hexane-extracted carinata presscake may supply less truly digested protein to the small intestine.

Based on the NRC model (Table 3.15.2), carinata meal showed higher AMCP (60 g/kg DM) and AECP (4.4 g/kg DM), but a lower ARUP (71 g/kg DM) compared with canola meal ($P<0.05$). Given the total metabolizable protein in the small intestine, which would contribute to the milk production, a higher MP value was observed in canola meal (153 g/kg DM vs. 136 g/kg DM). The degraded protein balance in carinata meal was higher than that in canola meal (259 g/kg DM vs. 140 g/kg DM). The hexane-extracted carinata presscake had a similar AMCP (62 g/kg DM) to carinata meal, and AECP of it was higher (4.5 g/kg DM). However, ARUP of hexane-extracted carinata presscake (26 g/kg DM) was the lowest among three co-products ($P<0.05$), consequently, resulting in its lower MP (92 g/kg DM) and feed milk value (1.9 kg milk/kg feed).

Table 3.14.1 Predicted truly absorbed protein supply to dairy cows and feed milk value of new carinata seeds (Yellow-AAC A110 vs. Brown-110915EM) in comparison with new canola seeds (Yellow-YN07 C1386 vs. Brown-N07 1374) and a commercial canola seed (Brown) using DVE/OEB system

Components	New Carinata Seeds (N_CN)		New Canola Seeds (N_CL)		Commercial Canola Seed (COMM)	SEM	P value	Contrast, P value		
	Yellow (AAC A110)	Brown (110915EM)	Yellow (YN07 C1386)	Brown (N07 1374)	Brown			N_CN vs N_CL	Yellow vs Brown	N_CN vs. COMM
Truly absorbed rumen-synthesised microbial protein in small intestine (g/kg DM)										
N_MCP	224^{a}	183^{b}	159^{b}	162^{c}	160^{c}	4.0	<0.001	<0.001	<0.001	<0.001
AMCP^{DVE}	41^{a}	38^{ab}	35^{b}	32^{c}	29^{c}	0.7	<0.001	<0.001	0.001	<0.001
Truly absorbed rumen-undegraded feed protein in small intestine (g/kg DM)										
BCP	81^{a}	77^{ab}	72^{ab}	77^{ab}	69^{b}	2.3	0.01	0.06	0.91	0.002
ABCP^{DVE}	30^{ab}	39^{a}	36^{a}	28^{ab}	24^{b}	2.6	0.01	0.36	0.95	0.003
Truly digested protein in small intestine (g/kg DM)										
DVE	68^{a}	69^{a}	67^{a}	51^{b}	43^{b}	3.2	<0.001	0.01	0.03	<0.001
Degraded protein balance (g/kg DM)										
OEB	160^{a}	123^{b}	104^{c}	112^{bc}	114^{bc}	3.5	<0.001	<0.001	0.001	<0.001
Feed milk value (kg milk/kg feed)	1.4^{a}	1.4^{a}	1.4^{a}	1.0^{b}	0.9^{b}	0.07	<0.001	0.01	0.03	<0.001

Notes: SEM: standard error of the mean. N_MCP: microbial protein synthesized in the rumen based on RDP; AMCP^{DVE}: truly absorbed microbial protein in the small intestine; BCP: rumen bypass feed crude protein; ABCP^{DVE}: truly absorbed bypass protein in the small intestine; DVE: truly digested protein in the small intestine; OEB: degraded protein balance. Means with different superscripts in the same row are significantly different according to Tukey method (P<0.05).

Table 3.14.2 Predicted truly absorbed protein supply to dairy cows and feed milk value of new carinata seeds (Yellow-AAC A110 vs. Brown-110915EM) in comparison with new canola seeds (Yellow-YN07 C1386 vs. Brown-N07 1374) and a commercial canola seed (Brown) using NRC Dairy model

Components	New Carinata Seeds (N_CN)		New Canola Seeds (N_CL)		Commercial Canola Seed (COMM)	SEM	P value	Contrast, P value		
	Yellow (AAC A110)	Brown (110915 EM)	Yellow (YN07 C1386)	Brown (N07 1374)	Brown			N_CN vs N_CL	Yellow vs Brown	N_CN vs. COMM
Truly absorbed rumen-synthesised microbial protein in small intestine (g/kg DM)										
MCP_{TDN}	164^{b}	152^{d}	171^{a}	160^{c}	164^{b}	0.7	<0.001	<0.001	<0.001	<0.001
$MCP_{RDP}{}^{NRC}$	197^{a}	162^{b}	142^{c}	144^{c}	142^{c}	3.5	<0.001	<0.001	<0.001	<0.001
$AMCP^{NRC}$	105^{a}	97^{b}	91^{c}	92^{bc}	91^{c}	1.4	<0.001	<0.001	0.045	<0.001
Truly absorbed rumen-undegraded feed protein in small intestine (g/kg DM)										
RUP^{NRC}	73^{a}	69^{ab}	65^{ab}	69^{ab}	62^{b}	2.0	0.01	0.06	0.91	0.002
$ARUP^{NRC}$	27^{ab}	35^{a}	33^{a}	25^{ab}	21^{b}	2.3	0.01	0.36	0.95	0.003
Truly digested rumen endogenous protein in small intestine (g/kg DM)										
ECP	11.5^{a}	11.4^{ab}	11.3^{bc}	11.2^{d}	11.3^{cd}	0.02	<0.001	<0.001	0.002	<0.001
$AECP^{NRC}$	4.58^{a}	4.57^{ab}	4.54^{bc}	4.50^{d}	4.52^{cd}	0.008	<0.001	<0.001	0.002	<0.001
Total truly absorbed protein in small intestine (g/kg DM)										
MP	137^{a}	137^{a}	128^{ab}	122^{bc}	116^{c}	2.5	<0.001	<0.001	0.26	<0.001
Degraded protein balance (g/kg DM)										
DPB	39^{a}	11^{b}	-35^{c}	-19^{c}	-27^{c}	4.7	<0.001	<0.001	0.23	<0.001
Feed milk value (kg milk/kg feed)	2.8^{a}	2.8^{a}	2.6^{ab}	2.5^{bc}	2.4^{c}	0.05	<0.001	<0.001	0.26	<0.001

Notes: SEM: standard error of the mean. MCP_{TDN}: microbial protein synthesized in the rumen based on TDN; $MCP_{RDP}{}^{NRC}$: microbial protein synthesized in the rumen based on available protein (0.85 of rumen degraded protein).

Table 3.14.2 Cont'd

$AMCP^{NRC}$: truly absorbed microbial protein in the small intestine; RUP^{NRC}: rumen undegraded feed crude protein; $ARUP^{NRC}$: truly absorbed rumen undegraded protein in the small intestine; ECP: rumen endogenous protein; $AECP^{NRC}$: truly absorbed rumen endogenous protein in the small intestine; MP: metabolizable protein; DPB: degraded protein balance. Means with different superscripts in the same row are significantly different according to Tukey method ($P<0.05$).

Table 3.15.1 Predicted truly absorbed protein supply to dairy cows and feed milk value of carinata meal and hexane-extracted carinata presscake in comparison with canola meal using DVE/OEB system

Components	Carinata Meal	Carinata Presscake	Canola Meal	SEM	P value
Truly absorbed rumen-synthesised microbial protein in small intestine (g/kg DM)					
N_MCP	357[b]	472[a]	217[c]	18.6	<0.001
$AMCP^{DVE}$	64[b]	78[a]	51[c]	1.5	<0.001
Truly absorbed rumen-undegraded feed protein in small intestine (g/kg DM)					
BCP	128[b]	64[c]	180[a]	5.8	<0.001
$ABCP^{DVE}$	79[b]	29[c]	108[a]	3.7	<0.001
Truly digested protein in the small intestine (g/kg DM)					
DVE	133[a]	101[b]	143[a]	2.8	<0.001
Degraded protein balance (g/kg DM)					
OEB	257[b]	350[a]	136[c]	16.3	<0.001
Feed milk value (kg milk/kg feed)	2.7[a]	2.0[b]	2.9[a]	0.06	<0.001

Notes: SEM: standard error of the mean. N_MCP: microbial protein synthesized in the rumen based on RDP; $AMCP^{DVE}$: truly absorbed microbial protein in the small intestine; BCP: rumen bypass feed crude protein; $ABCP^{DVE}$: truly absorbed bypass protein in the small intestine; DVE: truly digested protein in the small intestine; OEB: degraded protein balance. Means with different superscripts in the same row are significantly different according to Tukey method ($P<0.05$).

Table 3.15.2 Predicted truly absorbed protein supply to dairy cows and feed milk value of carinata meal and hexane-extracted carinata presscake in comparison with canola meal using NRC Dairy model

Components	Carinata Meal	Carinata Presscake	Canola Meal	SEM	P value
Truly absorbed rumen-synthesised microbial protein in small intestine (g/kg DM)					
MCP_{TDN}	94[a]	97[a]	80[b]	1.5	<0.001
MCP_{RDP}^{NRC}	314[b]	406[a]	199[c]	15.4	<0.001
$AMCP^{NRC}$	60[a]	62[a]	51[b]	0.9	<0.001
Truly absorbed rumen-undegraded feed protein in small intestine (g/kg DM)					
RUP^{NRC}	115[b]	57[c]	162[a]	5.2	<0.001
$ARUP^{NRC}$	71[b]	26[c]	97[a]	3.3	<0.001
Truly digested rumen endogenous protein in small intestine (g/kg DM)					
ECP	11.0[b]	11.2[a]	10.7[c]	0.02	<0.001
$AECP^{NRC}$	4.4[b]	4.5[a]	4.3[c]	0.01	<0.001
Total truly absorbed protein in small intestine (g/kg DM)					
MP	136[b]	92[c]	153[a]	2.9	<0.001
Degraded protein balance (g/kg DM)					
DPB	259[b]	364[a]	140[c]	16.6	<0.001
Feed milk value (kg milk/kg feed)	2.8[b]	1.9[c]	3.1[a]	0.06	<0.001

Notes: SEM: standard error of the mean. MCP_{TDN}: microbial protein synthesized in the rumen based on TDN; MCP_{RDP}^{NRC}: microbial protein synthesized in the rumen based on available protein (0.85 of rumen degraded protein); $AMCP^{NRC}$: truly absorbed microbial protein in the small intestine; RUP^{NRC}: rumen undegraded feed crude protein; $ARUP^{NRC}$: truly absorbed rumen undegraded protein in the small intestine; ECP: rumen endogenous protein; $AECP^{NRC}$: truly absorbed rumen endogenous protein in the small intestine; MP: metabolizable protein; DPB: degraded protein balance. Means with different superscripts in the same row are significantly different according to Tukey method ($P<0.05$).

3.4. Conclusions

In conclusion, the newly developed carinata seeds showed significant differences in chemical profiles compared with canola seeds, with higher CP and glucosinolates but lower oil content and fiber. The energy values were similar between carinata seeds and canola seeds with the same hull color. Higher rumen degraded feed CP and total digested protein were found in carinata seeds, but EDNDF and IDP were relatively lower. Yellow seeds had better seed quality because of their high CP, energy and digested protein supply than the brown. Carinata meal, higher in crude protein and energy, was advantageous in RDP supply for microbial protein synthesis in the rumen fermentation, but had lower IDP. It had the equivalent metabolizable protein supply compared to canola meal. The hexane-extracted carinata presscake had higher energy values and nutrient solubility; however, it inhibited RUP passing to the small intestine for absorption, and thus had a negative effect on the supply of truly absorbed protein in the small intestine. In general, the high level of glucosinolates of carinata seeds and carinata co-products would be an obstacle to their application in the feed industry.

Based on the nutritional study, carinata seeds and carinata co-products might be an alternative feed source, given their nutrient composition, energy values, rumen degradation and intestinal digestion features. However, cold pressing in this study significantly affected nutrient solubility and digestion, resulting in a negative effect on truly absorbed protein supply to dairy cattle. Further, the inherent structural differences between carinata vs. canola seeds or co-products remain undetected, since protein and carbohydrate structural characteristics are related to nutrient metabolism. Thus, further study is needed to investigate molecular structural differences, in order to reveal the relationship between molecular structural and nutritional quality of carinata seeds and co-products.

4. PROTEIN AND CARBOHYDRATE STRUCTURAL STUDY OF BRASSICA CARINATA SEEDS AND THE CO-PRODUCTS USING FOURIER TRANSFORM INFRARED VIBRATIONAL SPECTROSCOPY

4.1. Introduction

Chemical composition and inherent molecular structure of feeds determine nutrient bioavailability and metabolic features together. Fourier transform infrared (FTIR) vibrational spectroscopy is a rapid technology applied to detect how molecules and functional groups exist in a sample, which traditional chemical analysis fails to do. The mid-infrared spectrum (ca. 4,000-400 cm^{-1}) is used to identify molecular-level information, because strong absorbance of many molecules or functional groups can be observed in this region (Smith, 2011). The peak positions are determined by structures of molecules and functional groups in a sample, with peak intensities indicating the concentrations of molecules. FTIR vibrational spectroscopy has been utilized in rapid feed analysis and quality studies, with its advantage of detecting structural differences among various samples (liquid, solid and gas samples) (Kos et al., 2003; Sherazi et al., 2007; Udén, 2010), and molecular structural changes induced by different treatments (heating, transgenetic techniques or pelleting) (Xin et al., 2014b; Peng et al., 2014a; Li et al., 2015; Huang et al., 2015b). It is considered accurate and sensitive for small amount of samples. In addition, structural features of protein and carbohydrate molecules are related to digestion characteristics, and therefore may be used to predict nutrient bioavailability (Peng et al., 2014b; Yu and Nuez-Ortín, 2010). In order to reveal structural features of carinata samples in comparison with canola seeds and co-products, the objectives were (1) to detect protein and carbohydrate structure of carinata seeds and the co-products, (2) to determine the effect of cold pressing on molecular structural characteristics of carinata, and (3) to investigate the relationship between molecular structure spectral features and nutrient bioavailability of *Brassica carinata* and carinata co-products.

4.2. Materials and Methods

4.2.1. Sample Preparation and Spectra Collection

The newly developed yellow and brown carinata seeds (Yellow-AAC A110 and Brown-110915EM), new yellow and brown canola seeds (Yellow-YN07 C1386 and Brown-N07 1374) and seeds of a commercial brown canola variety were used in this study, with canola seeds as the reference samples. Carinata meal and hexane-extracted carinata presscake were also compared to canola meal. All samples were ground by a coffee grinder (PC770, Loblaws Inc., Toronto, ON) for 20 seconds. Fourier transform infrared (FTIR) vibrational spectroscopy, equipped with a JASCO FT/IR-4200 spectroscope (JASCO Corp., Tokyo, Japan), a ceramic infrared light source and a deuterated L-alanine doped triglycine sulfate detector consisting of an MIRacle attenuated total reflectance (ATR) accessory module and a zinc selenium (ZnSe) crystal and pressure clamp (Pike Technologies, Madison, WI, US), was used at the spectroscopy lab of Department of Animal and Poultry Science, University of Saskatchewan (Saskatoon, SK). The spectra were collected in the mid-infrared region (ca. 4000-700 cm^{-1}) (Figure 4.1.1 (a)) with a spectral resolution of 4 cm^{-1} and 128 co-added scans using JASCO Spectra Manager II Software; and background spectra were corrected with 256 co-added scans for each sample. Five replicate spectra of each sample were collected and analysed by OMNIC 7.3 Software (Thermo Electron Corp., Madison, WI, US). The structural spectral information of protein, structural CHO, cellulosic compounds and total carbohydrate was identified by analyzing absorption peak parameters (baseline, region, peak height and area according to Huang (2015).

4.2.2. Univariate Spectral Analyses of Protein and Carbohydrate Structure

The univariate spectral analyses of protein-relative functional groups included amide I absorption peak areas (ca. 1729-1573 cm^{-1}) and heights (ca. 1647 cm^{-1}), amide II absorption peak

areas (ca. 1573-1479 cm^{-1}) and heights (ca. 1537 cm^{-1}), secondary structure α-helix peak heights (ca. 1652 cm^{-1}) and β-sheet peak heights (ca. 1629 cm^{-1}) (Figure 4.1.1). The detection of protein secondary structure relied on the Second Derivative function or the Fourier Self-deconvolution (FSD) function in OMNIC 7.3 Software (Yu, 2006, 2010). Afterwards, the height and area ratios of amide I to amide II and height ratios of α-helix to β-sheet were calculated.

Structural carbohydrate (Figure 4.1.2), cellulosic compounds (Figure 4.1.3) and total carbohydrate (Figure 4.1.4) were estimated for carbohydrate-related functional groups of carinata and canola samples. For structural CHO, the absorption peak heights of three main structural CHO were detected at 1415 cm^{-1}, 1373 cm^{-1} and 1234 cm^{-1} approximately, with the baselines set at the range of 1479-1182 cm^{-1}. Cellulosic compounds were located at the region of structural CHO, however, detected with the baselines ranging from 1307 cm^{-1} to 1182 cm^{-1} and peak centers at 1234 cm^{-1} approximately. As for total CHO, the area regions ranged from 1192 cm^{-1} to 881 cm^{-1} approximately. The areas of three major peaks were at ca. 1192-1130 cm^{-1}, 1130-1090 cm^{-1} and 1090-881 cm^{-1}, with peak heights located at ca. 1154 cm^{-1}, 1105 cm^{-1} and 1050 cm^{-1}, respectively. Area ratios of three major CHO were calculated, and structure spectral features of new carinata seeds and the co-products were compared to canola seeds and canola meal using SAS 9.3.

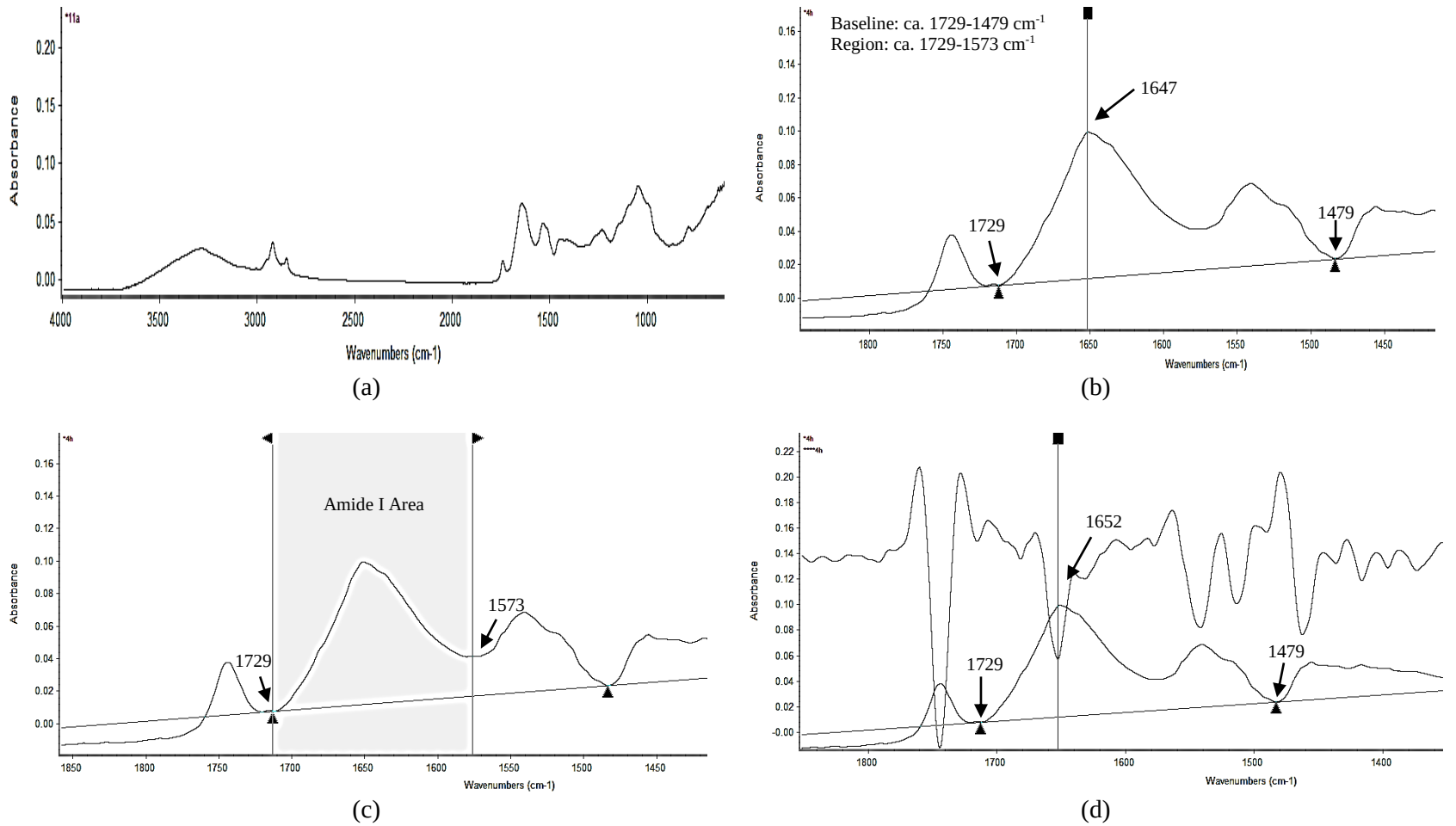
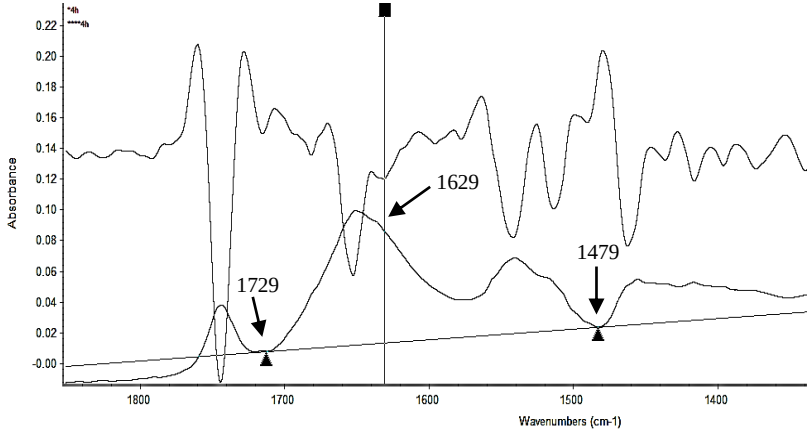
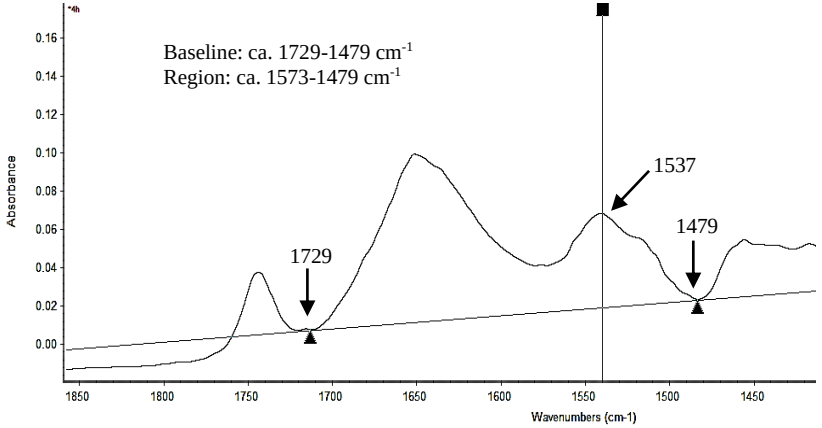


Figure 4.1.1 Fourier transform infrared (FTIR) spectroscopy spectral analyses. (a) whole spectrum region (ca. 4,000-700 cm^{-1}); Spectral parameters of protein structure (baseline region: ca. 1729-1479 cm^{-1}) consisting of: (b) amide I peak height (ca. 1647 cm^{-1}); (c) amide I area (ca. 1729-1573 cm^{-1}); (d) protein secondary structure: α-helix peak height (ca. 1652 cm^{-1}); (e) protein secondary structure: β-sheet peak height (ca. 1629 cm^{-1}); (f) amide II peak height (ca. 1537 cm^{-1}); and (g) amide II area (ca. 1573-1479 cm^{-1})



(e)



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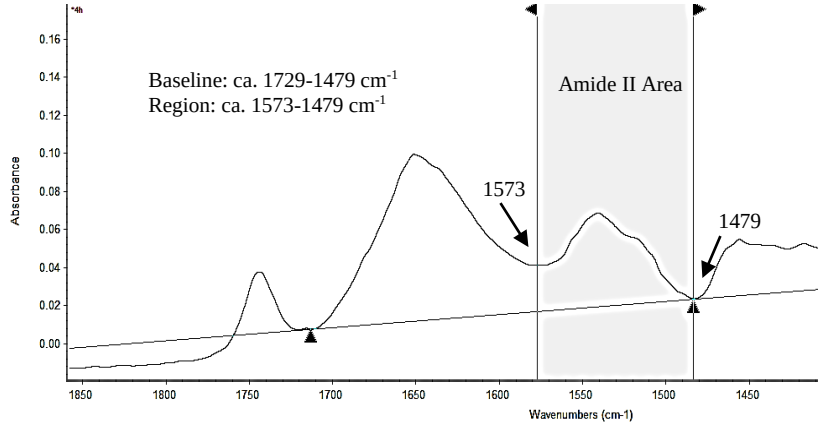


(g)

Figure 4.1.1 Cont'd

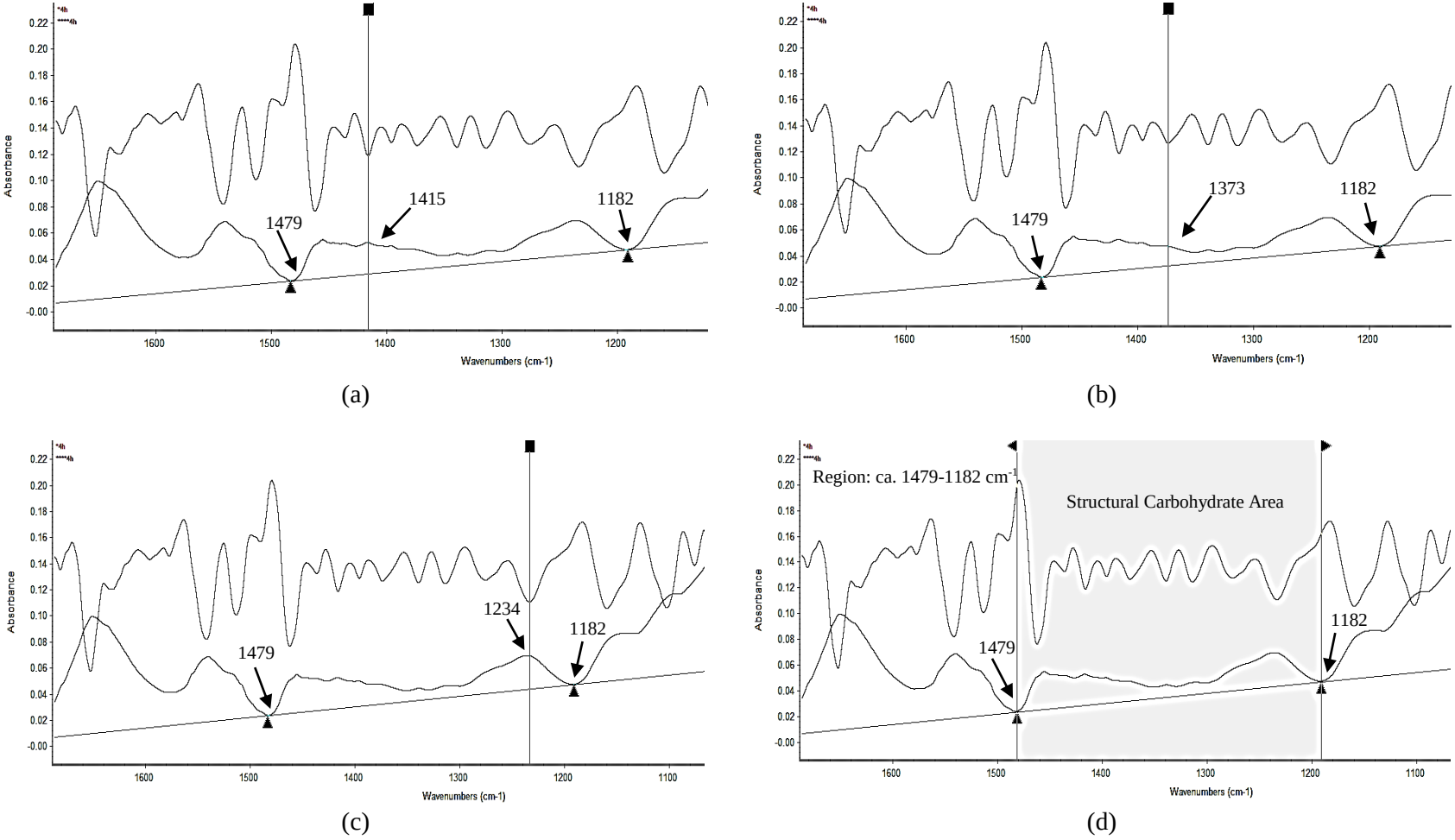
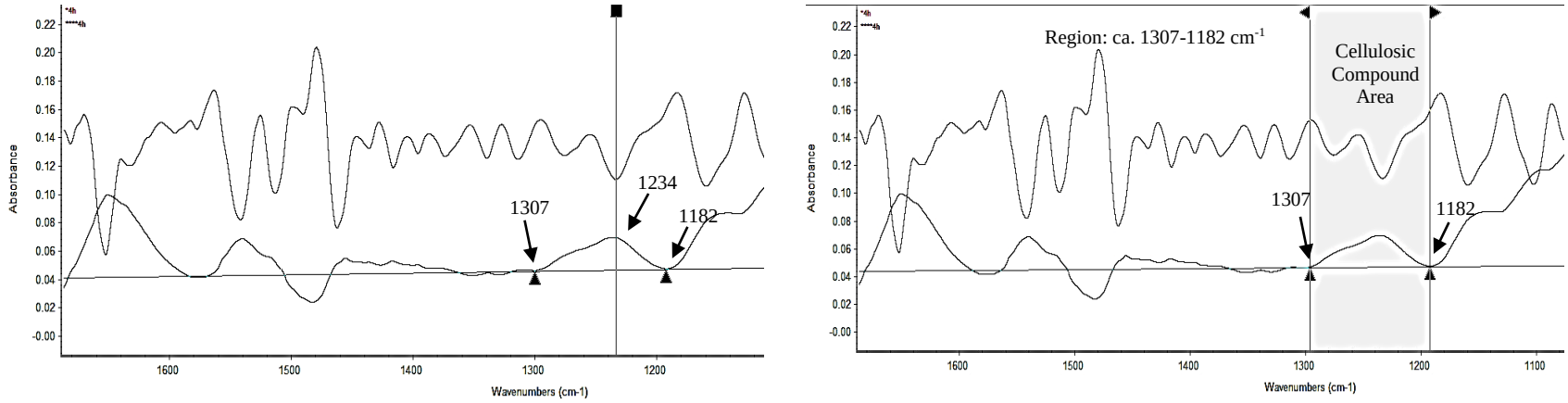


Figure 4.1.2 Fourier transform infrared (FTIR) spectroscopy spectral analyses. Spectral parameters of structural carbohydrate structure (baseline region: ca. 1479-1182 cm^{-1}) consisting of: (a) 1st peak height (ca. 1415 cm^{-1}); (b) 2nd peak height (ca. 1373 cm^{-1}); (c) 3rd peak height (ca. 1234 cm^{-1}); and (d) total area (ca. 1479-1182 cm^{-1})



(a) (b)

Figure 4.1.3 Fourier transform infrared (FTIR) spectroscopy spectral analyses. Spectral parameters of cellulosic compound structure (baseline region: ca. 1307-1182 cm^{-1}) consisting of: (a) peak height (ca. 1234 cm^{-1}); and (b) area (ca. 1307-1182 cm^{-1})

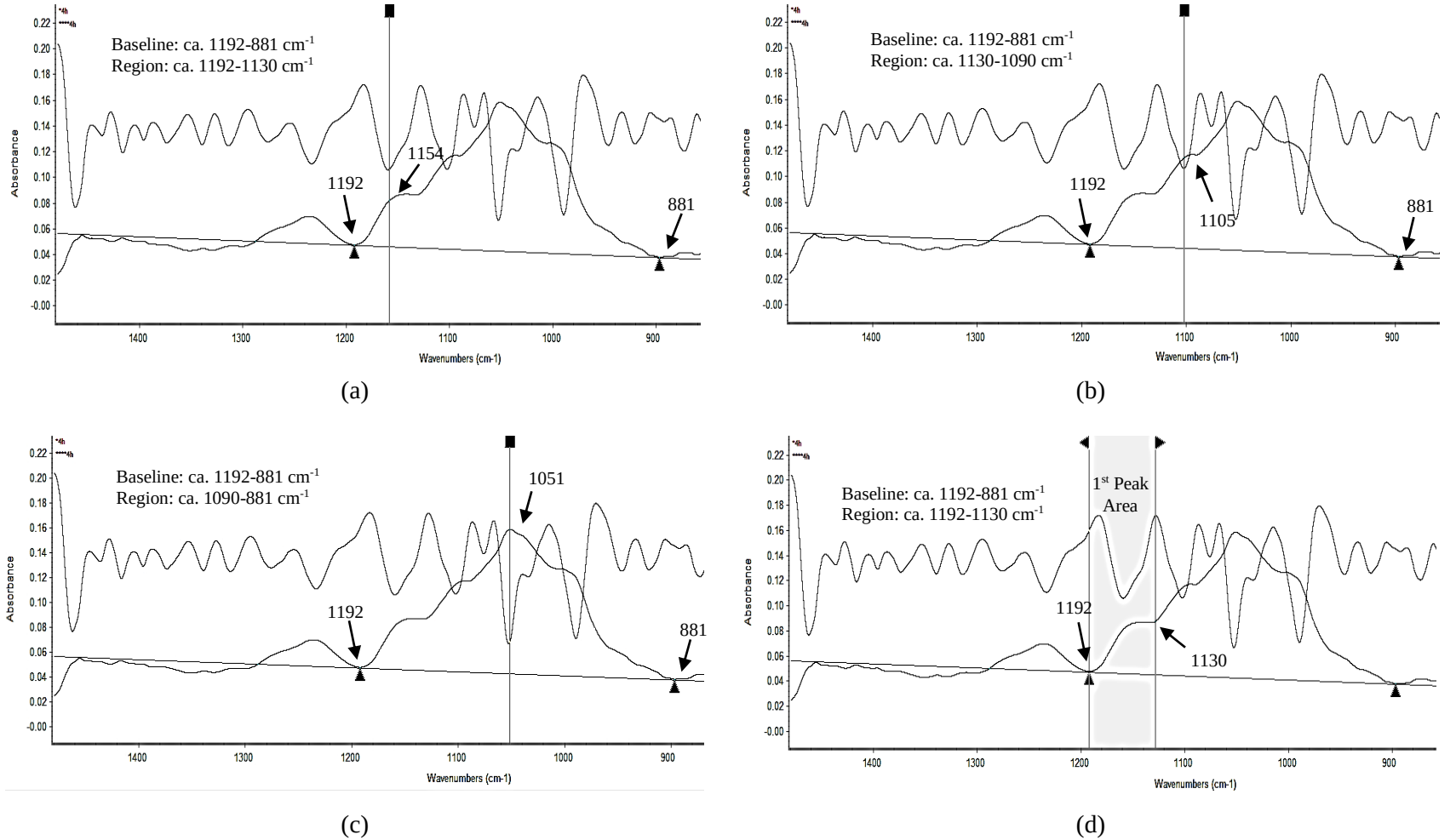
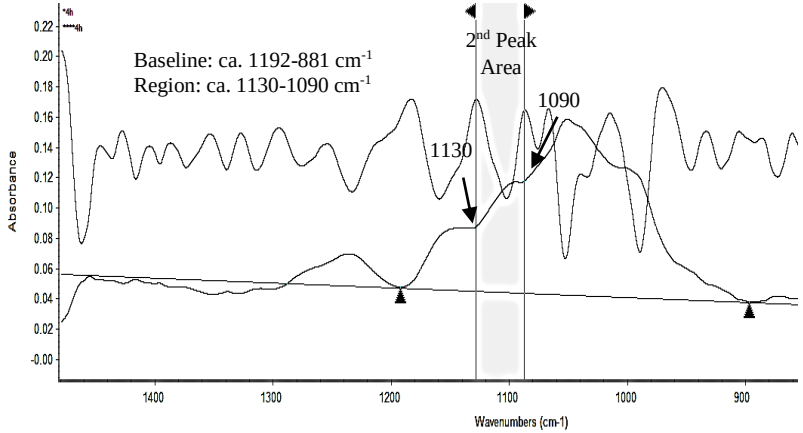


Figure 4.1.4 Fourier transform infrared (FTIR) spectroscopy spectral analyses. Spectral parameters of total carbohydrate structure (baseline region: ca. 1192-881 cm^{-1}) consisting of: (a) 1st peak height (ca. 1154 cm^{-1}); (b) 2nd peak height (ca. 1105 cm^{-1}); (c) 3rd peak height (ca. 1051 cm^{-1}); (d) 1st peak area (ca. 1192-1130 cm^{-1}); (e) 2nd peak area (ca. 1130-1090 cm^{-1}); (f) 3rd peak area (ca. 1090-881 cm^{-1}); and (g) total area (ca. 1192-881 cm^{-1})



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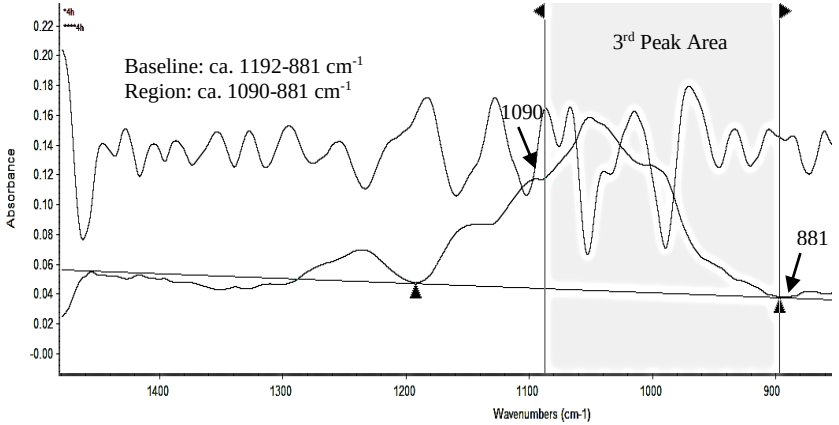

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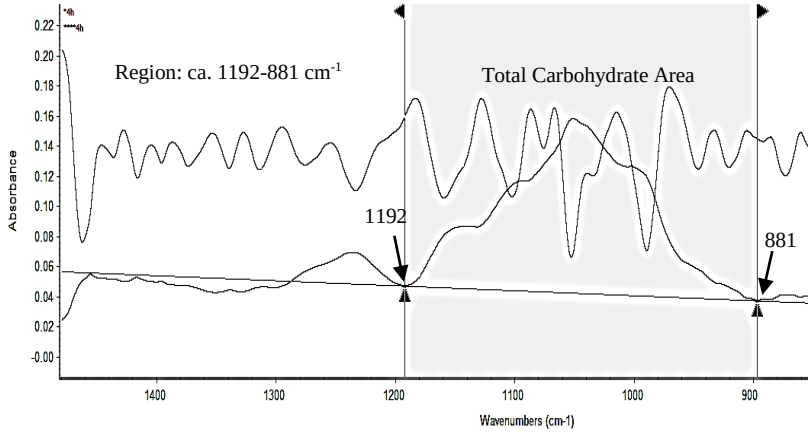


(g)

Figure 4.1.4 Cont'd

4.2.3. Multivariate Spectral Analyses

Multivariate spectral analyses were conducted by the Agglomerative Hierarchical Cluster Analysis (CLA) and the Principal Component Analysis (PCA) for all seed and co-product samples using Statistica 8.0 Software (StatSoft Inc., Tulsa, OK, USA). For the cluster analyses, Ward's Algorithm method was used for clustering calculation, with results displayed as dendrograms (Yu, 2010), and the Euclidean method was applied to the distance matrix calculation. In the Principal Component Analysis, all original variables were transferred to a set of new uncorrelated variables called principal components (PCs). Then, the first principal component (PC1) and second principal component (PC2) were generated in a scatter plot and used to describe all variables. The specific spectral analyses of these two methods were explained in Yu (2005a, 2010). The data of functional groups in the regions of protein (ca. 1729-1479 cm^{-1}), structural CHO (ca. 1479-1182 cm^{-1}) and total CHO (ca. 1192-881 cm^{-1}) were analyzed by these multivariate spectral analyses.

4.2.4. Statistical Analyses

The statistical analyses of protein and carbohydrate structure spectral data were conducted using the MIXED procedure of SAS 9.3 (SAS Institute, Inc., Cary, NC, US) with the model of $Y_{ij} = \mu + F_i + e_{ij}$, where Y_{ij} is the observation of the dependent variable ij, μ is the population mean of the variable, F_i is the effect of different feedstocks as a fixed effect (different sources were treated as replication), and e_{ij} is the random error associated with the observation ij. Contrast statements were used for all seed samples to compare the differences between new carinata seeds and new canola seeds, new carinata seeds and the commercial canola seed, yellow and brown seeds. Means were compared with significance declared at $P<0.05$.

The relationships between protein and carbohydrate structure spectral parameters and protein-related chemical composition, rumen degradation and intestinal digestion characteristics,

and predicted protein supply using the DVE/OEB system were revealed by correlation and regression analyses in SAS 9.3. The protein-related spectral parameters included peak height and area of amide I, peak height and area of amide II, height and area ratios of amide I to amide II, height of α-helix, height of β-sheet, and height ratio of α-helix to β-sheet. The CHO-related spectral parameters contained heights of the three main peaks in the structural CHO region, area of structural CHO region, peak height and area of cellulosic components, heights and areas of the three main peaks in total CHO region, and area of total CHO region. After checking normality of the data for correlation study, rank correlation with the SPEARMAN option was applied into the PROC CORR procedure of SAS 9.3. In order to select relative variables of spectral parameters for predicting nutritional values, multiple regression study was conducted using PROC REG procedure in SAS 9.3 with the model of $Y = a + b_1 \times x_1 + b_2 \times x_2 + \ldots + b_n \times x_n$. By means of the STEPWISE option and "SLENTRY = 0.05, SLSTAY = 0.05", variables, which were significant at $P<0.05$, were left in the prediction models. Afterwards, the UNIVARIATE procedure in SAS 9.3 with Normal and PLOT options was used for residual analysis. Additionally, collinearity was detected by the VIF option to eliminate the influence of correlated dependent variables.

4.3. Results and Discussion

4.3.1. Protein Structure Spectral Features

4.3.1.1. Protein Structure Spectral Features of Carinata Seeds in Comparison with Canola Seeds

Table 4.1 shows the protein structure spectral characteristics of carinata and canola seeds detected by FTIR. In the protein structure region (ca. 1722-1483 cm^{-1}), new carinata seeds had higher amide I and amide II peaks than canola seeds (contrast $P<0.05$), though there was no significant difference found in the height ratios of amide I to amide II among all seeds. However, given the contrast results, carinata seeds were relatively higher in height ratios of amide I to amide

II than new canola seeds and the commercial seed ($P<0.05$). Amongst all seed samples, they were similar in amide I and amide II areas, except that carinata seeds were significantly different from new canola seeds ($P<0.05$). The differences in area ratios of amide I to amide II were not distinguishable between carinata seeds vs. new canola seeds, and carinata seeds vs. commercial canola seed (contrast $P>0.05$). For protein secondary structure, carinata seeds had higher peaks of α-helix and height ratios of α-helix to β-sheet than canola seeds ($P<0.05$) but had similar β-sheet peak height. However, Xin et al. (2013c) found carinata seeds had significantly different amide I area and β-sheet peak height from canola seeds. Seed coat colors did not significantly affect protein structure features of the new *Brassica carinata* in our study, while previous studies of yellow carinata seed showed higher amide I area, amide II peak height and β-sheet peak height than the brown (Xin et al., 2013c).

New carinata seeds were compared with new canola seeds and the commercial canola seed at the protein structure region (ca. 1722-1483 cm^{-1}) by multivariate spectral analyses (Figure 4.2). No separate clusters were distinguished by CLA between new carinata seeds and new canola seeds [Figure 4.2 (1)], or between new carinata seeds and the commercial canola seed [Figure 4.2 (3)]. This was supported by the results from PCA, with overlapped ellipse groups observed for carinata seeds and canola seeds [Figure 4.2 (2), (4)]. The first two principal components (PCs) represented 86.21% and 10.54% of the variation in the protein structure of new carinata seeds and new canola seeds, 90.88% and 5.84% of the variance for new carinata seeds and the commercial canola seed. Similarly, mixed cluster dengrograms and non-separate ellipses were found among carinata and canola seeds of previous varieties (Xin et al., 2013c). This may reflect a similarity in protein structural make-up within the entire protein structural region among the new carinata seeds and canola seeds.

4.3.1.2. Protein Structure Spectral Features of Carinata Meal and Hexane-extracted Carinata Presscake in Comparison with Canola Meal

Carinata meal and canola meal were similar in amide I peak heights and areas, amide II peak heights and areas, area ratios of amide I to amide II, and protein secondary structure (α-helix peak height and β-sheet peak height) ($P \geq 0.05$), as Table 4.2 shows. However, carinata meal was significantly higher in the height ratio of amide I to amide II (1.75 vs. 1.63) and height ratio of α-helix to β-sheet (1.16 vs. 1.04) ($P < 0.05$) than canola meal. These were different from results reported by Xin and Yu (2013a) that carinata meal had relatively higher amide I peak height (0.041 vs. 0.033), amide I area (3.245 vs. 2.693), α-helix height (0.042 vs. 0.032) and β-sheet height (0.037 vs. 0.030) ($P < 0.05$). Theodoridou and Yu (2013a) observed higher amide II area, α-helix peak height and β-sheet peak height but lower area ratio of amide I to amide II in canola presscake than canola meal. In our study, without heating involved, hexane-extracted carinata presscake had significantly lower absorption intensities than carinata meal in protein structure region (ca. 1729-1479 cm^{-1}) ($P < 0.05$), except for similar height and area ratios of amide I to amide II as well as β-sheet height ($P \geq 0.05$). Yu (2005b) reported that the percentage of β-sheet may be negatively correlated with protein digestion. This may partially explain the higher TDP of hexane-extracted carinata presscake compared to canola meal, due to the lower β-sheet height (0.038 vs. 0.050).

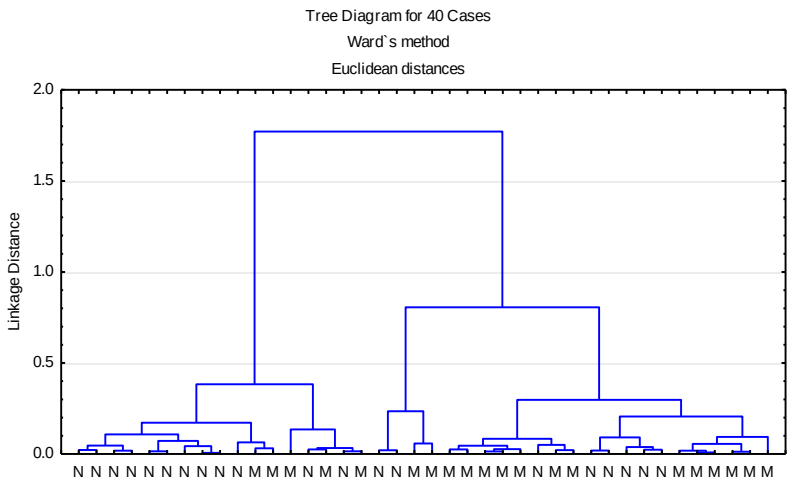
Results from multivariate analysis, CLA and PCA, are shown in Figure 4.3. The comparisons between carinata meal and canola meal [Figure 4.3 (1), (2)] reflected similar protein structural features within the entire protein-related region, because of mixed cluster classes and overlapped ellipses, with 75.09% and 13.29% of the total variance explained in PCA results. The hexane-extracted carinata presscake class was not fully separated from carinata meal class in the cluster analysis at the protein structure region (ca. 1729-1479 cm^{-1}), as well they did not group into two separated ellipses in the PCA plot, indicating similar protein structure between carinata meal

and extracted-presscake given all data from the entire protein structural spectral region [Figure 4.3 (3), (4)]. The PC1 and PC2 represented 90.36% and 8.57% of the variation in the protein structure of carinata meal and presscake. The hexane-extracted carinata presscake had distinct clusters below a linkage distance of 0.7 from canola meal in CLA, and the PCA plot showed two distinguishable ellipses in which PC1 and PC2 explained 93.82% and 5.47% of the variation respectively [Figure 4.3 (5), (6)]. This may indicate hexane-extracted carinata presscake had different protein structural features compared with canola meal at the whole region.

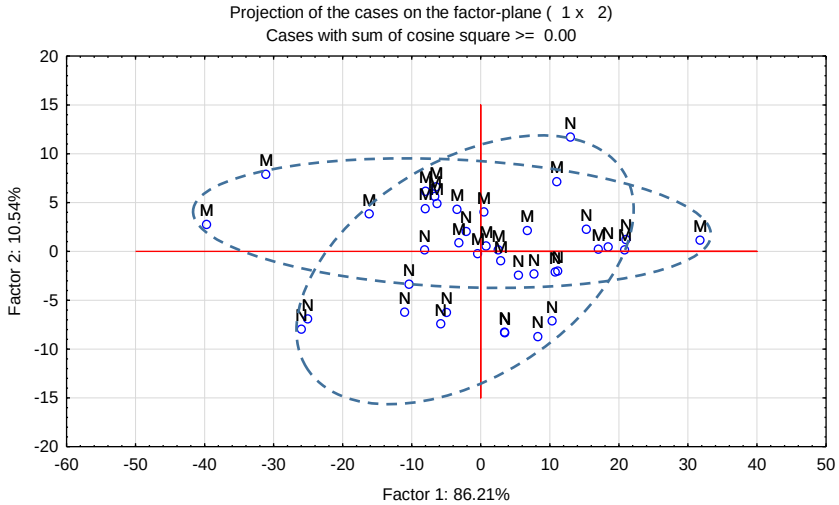
Table 4.1 Protein structure spectral characteristics of new carinata seeds (Yellow-AAC A110 vs. Brown-110915EM) in comparison with new canola seeds (Yellow-YN07 C1386 vs. Brown-N07 1374) and a commercial canola seed (Brown) using FTIR vibrational spectroscopy

Components	Peak region and center (cm^{-1})	New Carinata Seeds (N_CN)		New Canola Seeds (N_CL)		Commercial Canola Seed (COMM)	SEM	P value	Contrast, P value		
		Yellow (AAC A110)	Brown (110915 EM)	Yellow (YN07 C1386)	Brown (N07 1374)	Brown			N_CN vs N_CL	N_CN vs. COMM	Yellow vs Brown
Protein primary structure											
Amide I peak height	~1651	0.083^{a}	0.078^{ab}	0.071^{b}	0.070^{b}	0.072^{ab}	0.0028	0.01	0.001	0.02	0.39
Amide II peak height	~1541	0.047^{a}	0.045^{ab}	0.041^{b}	0.040^{b}	0.042^{b}	0.0017	0.03	0.003	0.047	0.52
Height ratio of Amide I to II		1.76	1.76	1.74	1.72	1.73	0.013	0.11	0.02	0.04	0.39
Amide I area	1722-1577	5.73	5.55	5.09	4.99	5.12	0.216	0.08	0.01	0.06	0.51
Amide II area	1577-1483	2.55	2.40	2.17	2.25	2.26	0.103	0.09	0.01	0.09	0.70
Area ratio of Amide I to II		2.25^{ab}	2.32^{ab}	2.35^{a}	2.22^{b}	2.27^{ab}	0.029	0.02	0.97	0.67	0.34
Protein secondary structure											
α-helix peak height	~1653	0.082^{a}	0.078^{ab}	0.070^{ab}	0.066^{b}	0.067^{b}	0.0035	0.01	0.002	0.01	0.26
β-sheet peak height	~1631	0.068	0.063	0.061	0.062	0.064	0.0030	0.47	0.20	0.61	0.43
Height ratio of α-helix to β-sheet		1.20^{ab}	1.27^{a}	1.15^{ab}	1.07^{b}	1.06^{b}	0.042	0.004	0.01	0.002	0.85

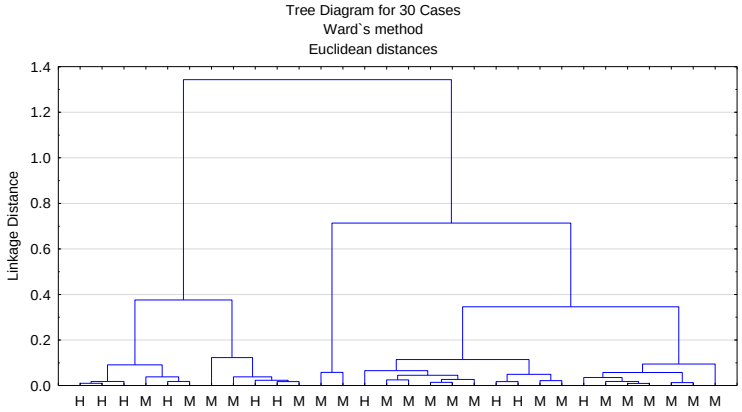
Notes: SEM: standard error of the mean. Means with different superscripts in the same row are significantly different according to Tukey method ($P<0.05$).



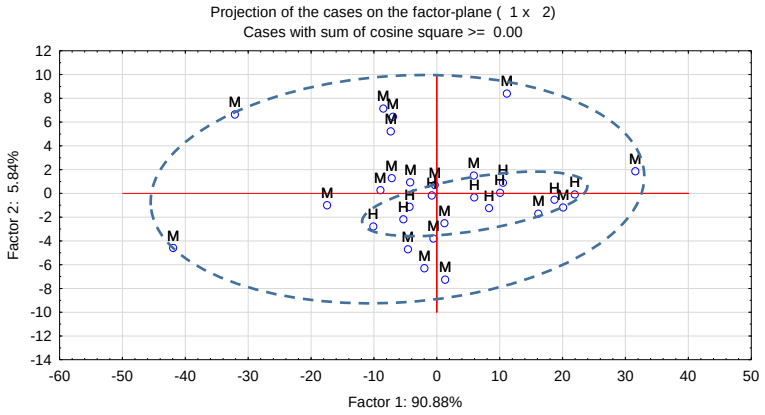
(1) CLA Comparison: Carinata Seeds (M) & Canola Seeds (N)



(2) PCA Comparison: Carinata Seeds (M) & Canola Seeds (N)



(3) CLA Comparison: Carinata Seeds (M) & Commercial Canola Seeds (H)



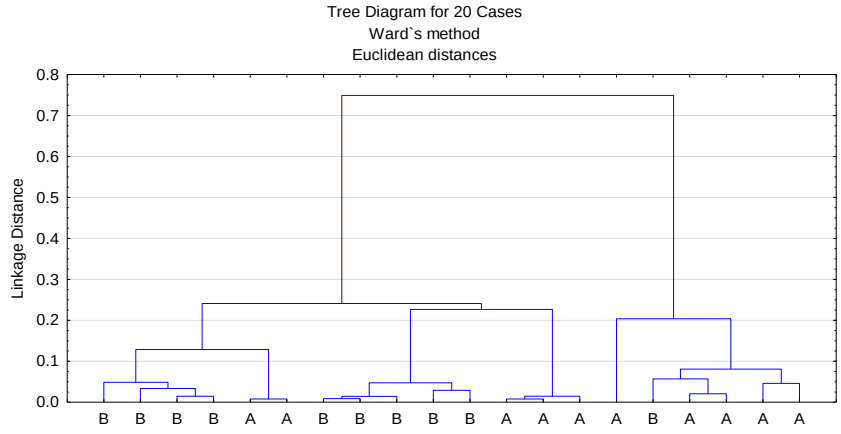
(4) PCA Comparison: Carinata Seeds (M) & Commercial Canola Seeds (H)

Figure 4.2 Multivariate spectral analyses of new carinata seeds (Yellow-AAC A110 and Brown-110915EM) in comparison with new canola seeds (Yellow-YN07 C1386 and Brown-N07 1374) and commercial canola seed (Brown) using FTIR vibrational spectroscopy at protein structure region (ca. 1722-1483 cm^{-1}). CLA (cluster analysis): cluster method (Ward's algorithm) and distance method (Euclidean). PCA (principal component analysis): Scatter plots of the 1st principal components (PC1) vs. the 2nd principal components (PC2)

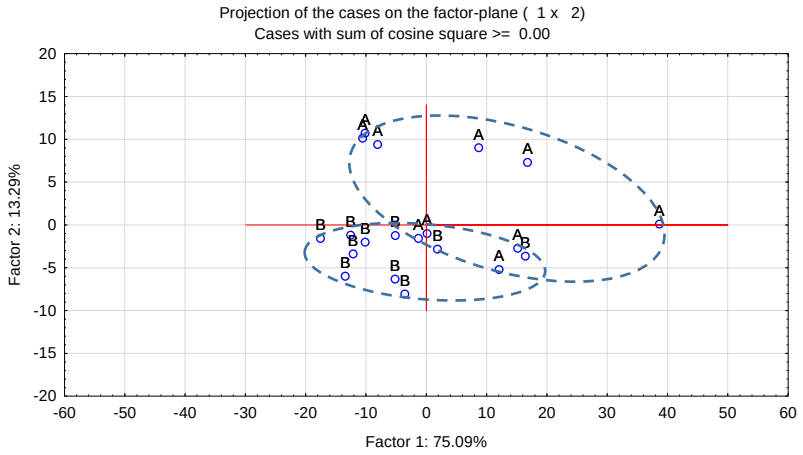
Table 4.2 Protein structure spectral characteristics of carinata meal and hexane-extracted carinata presscake in comparison with canola meal using FTIR vibrational spectroscopy

Components	Peak region and center (cm^{-1})	Carinata Meal	Carinata Presscake	Canola Meal	SEM	P value
Protein primary structure						
Amide I peak height	~1647	0.051^{a}	0.043^{b}	0.053^{a}	0.0023	0.01
Amide II peak height	~1537	0.029^{a}	0.024^{b}	0.033^{a}	0.0014	<0.001
Height ratio of Amide I to II		1.75^{a}	1.76^{a}	1.63^{b}	0.012	<0.001
Amide I area	1729-1573	4.02^{a}	3.31^{b}	4.51^{a}	0.168	<0.001
Amide II area	1573-1479	1.69^{a}	1.40^{b}	1.80^{a}	0.077	0.001
Area ratio of Amide I to II		2.38^{ab}	2.37^{b}	2.50^{a}	0.034	0.02
Protein secondary structure						
α-helix peak height	~1652	0.051^{a}	0.042^{b}	0.052^{a}	0.0022	0.004
β-sheet peak height	~1629	0.044^{ab}	0.038^{b}	0.050^{a}	0.0020	0.001
Height ratio of α-helix to β-sheet		1.16^{a}	1.10^{b}	1.04^{c}	0.009	<0.001

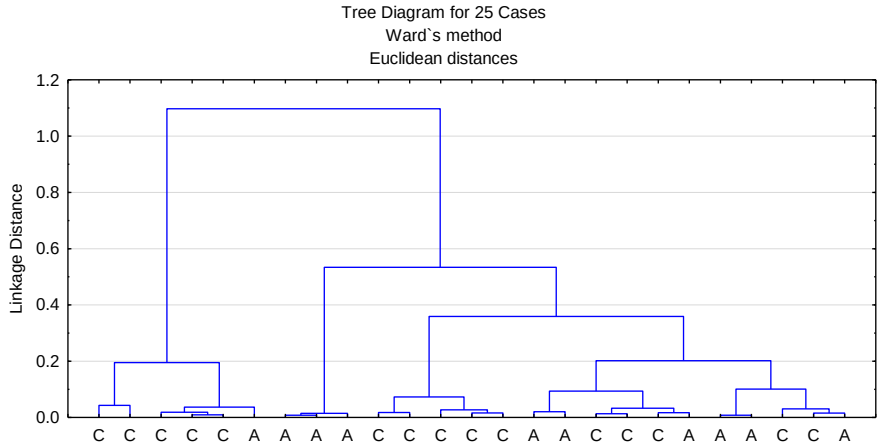
Notes: SEM: standard error of the mean. Means with different superscripts in the same row are significantly different according to Tukey method ($P<0.05$).



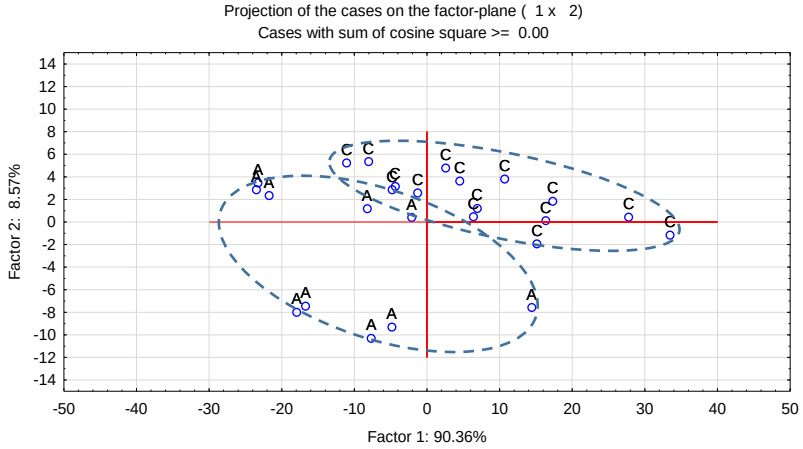
(1) CLA Comparison: Carinata Meal (A) and Canola Meal (B)



(2) PCA Comparison: Carinata Meal (A) and Canola Meal (B)

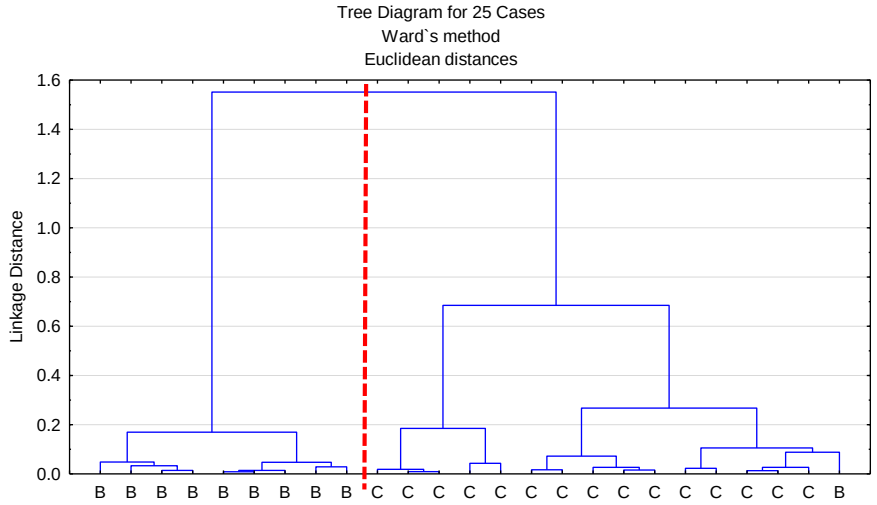


(3) CLA Comparison: Carinata Meal (A) and Carinata Presscake (C)

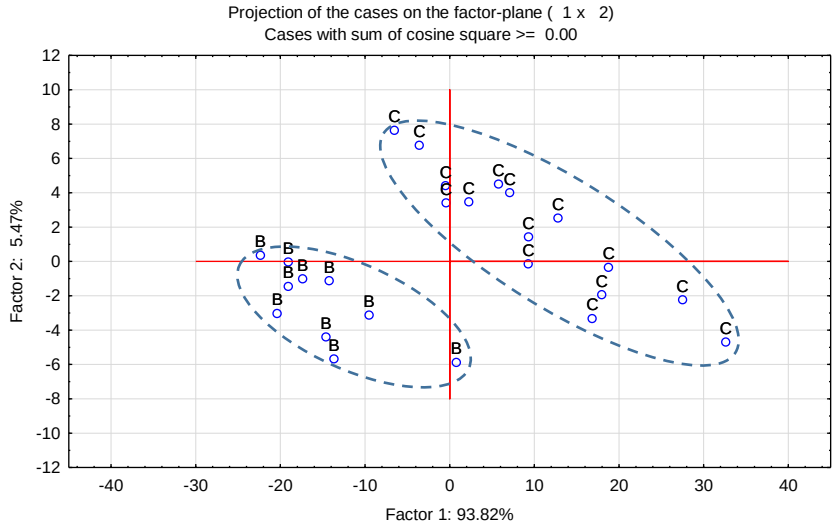


(4) PCA Comparison: Carinata Meal (A) and Carinata Presscake (C)

Figure 4.3 Multivariate spectral analyses of carinata meal (A) and hexane-extracted carinata presscake (C) in comparison with canola meal (B) using FTIR vibrational spectroscopy at protein structure region (ca. 1729-1479 cm^{-1}). CLA (cluster analysis): cluster method (Ward's algorithm) and distance method (Euclidean). PCA (principal component analysis): Scatter plots of the 1st principal components (PC1) vs. the 2nd principal components (PC2)



(5) CLA Comparison: Canola Meal (B) and Carinata Presscake (C)



(6) PCA Comparison: Canola Meal (B) and Carinata Presscake (C)

Figure 4.3 Cont'd

4.3.2. Carbohydrate Structure Spectral Features

4.3.2.1. Carbohydrate Structure Spectral Features of Carinata Seeds in Comparison with Canola Seeds

Carbohydrate (CHO) structure spectral features of new carinata and canola seeds, including structural CHO, cellulosic compounds and total CHO, are shown in Table 4.3. Structural CHO (STCHO) contains cellulosic and hemi-cellulosic compounds, and non-structural CHO includes starch and sugar. Cellulosic compounds (CELC) consist of phenolic-carbohydrate complexes, hemicellulose encrustation and cellulose crystallinity (Yang, 2012).

The heights of 2nd and 3rd peaks at the structural CHO region (ca. 1483-1193 cm^{-1}) were significantly different between new carinata seeds and canola seeds (contrast $P<0.05$), but there was no difference found in STCHO 1st peak height. New carinata seeds were higher in STCHO area than the commercial canola seed (contrast $P<0.05$), but similar to new canola seeds. Yellow carinata seed had lower 1st and 2nd peak heights than the brown carinata seed. According to the multivariate spectral analyses at the whole STCHO region (Figure 4.4.1), carinata seeds were not fully distinguishable from new canola seeds or commercial canola seed, given the mixed cluster groups and two overlapped ellipses on CLA and PCA plots respectively. This indicates similar structural carbohydrate structural features of carinata and canola seeds with respect to the whole STCHO spectrum. For the cellulosic compounds (ca. 1304-1193 cm^{-1}), both carinata seeds showed significantly higher peak height and area versus canola seeds ($P<0.05$). Hull color (yellow or brown) did not have a significant impact on CELC structural spectral characteristics of carinata seeds (1.12 vs. 1.15). The three main absorption peaks of total CHO were obtained at the region of ca. 1193-883 cm^{-1}. Carinata seeds were lower in total CHO 1st peak areas, total CHO 3rd peak areas and total CHO areas relative to new canola seeds, but higher in total CHO 2nd peak heights (contrast $P<0.05$). Moreover, the differences in total CHO three peak heights and 1st peak areas

were significant between newly developed carinata seeds and commercial canola seed (contrast $P<0.05$). No significant difference was found for total CHO 2nd peak areas ($P>0.05$) among all seeds. Comparing seeds with different coat colors, yellow cultivars were distinct from the brown in total CHO 1st peak heights and areas (contrast $P<0.05$). Within the total CHO functional groups spectrum, carinata seeds and new canola seeds did not form two independent clusters in CLA or two divided ellipses in PCA with 70.76% and 18.99% of the variance explained in the plot (Figure 4.4.2). Carinata seeds were likewise not distinguishable from the commercial canola seed though 81.55% of the variation could be represented by the first principal components. Regarding the area ratios, carinata seeds had the highest area ratios of STCHO to total CHO as well as area ratios of CELC to total CHO but lowest area ratios of STCHO to CELC.

Another study reported that carinata seeds had similar STCHO 1st peak heights, STCHO areas, total CHO 1st and 3rd peak heights as well as total CHO 3rd peak areas compared to canola seeds (Xin et al., 2013d). Also, seeds with different coat colors were not significantly different in STCHO 1st peak heights, total CHO 2nd and 3rd peak areas, area ratios of STCHO to CELC, area ratios of STCHO to total CHO, and area ratios of CELC to total CHO ($P>0.05$).

4.3.2.2. Carbohydrate Structure Spectral Features of Carinata Meal and Hexane-extracted Carinata Presscake in Comparison with Canola Meal

The spectral features of carbohydrate-related functional groups were detected by ATR-FTIR for three co-products (Table 4.4). Carinata meal had relatively lower structural CHO peak heights (0.011, 0.005, 0.012) and area (1.96) than canola meal (0.017, 0.011, 0.012 for three peaks; 2.77 for area) ($P<0.05$), with the exception of similar 3rd peak height in the STCHO region (ca. 1479-1182 cm^{-1}). The hexane-extracted carinata presscake differed from carinata meal in STCHO 1st and 2nd peak heights (0.008 and 0.004) as well as STCHO area (1.65) ($P<0.05$). The comparison of STCHO structure using data from the whole STCHO spectrum was conducted by CLA and

PCA (Figure 4.5.1). To describe the spectral characteristics of functional groups, univariate spectral analysis uses absorption peak height and area, which are obtained from a specific spectral region. Conversely, all data from the entire spectral region are applied to multivariate spectral analyses (CLA and PCA). Carinata meal was not clearly distinct from canola meal in regard to mixed clusters under the linkage distance of 0.4. As well, the hexane-extracted carinata presscake was not fully distinguished from carinata meal although 98.05% and 1.35% of the variability could be explained by PC1 and PC2. However, it formed a separated ellipse from canola meal in the PCA plot and mixed cluster groups with canola meal under the linkage distance of 0.8.

Three co-products were similar regarding spectral features within cellulosic compounds region (ca. 1307-1182 cm^{-1}) in univariate analysis (Table 4.4). In the total carbohydrate region (ca. 1192-881 cm^{-1}), the three main peaks were located at approximately 1154 cm^{-1}, 1105cm^{-1} and 1051 cm^{-1} on the spectrum. Canola meal had the highest absorption intensities in this region, followed by carinata meal. The total CHO 3rd peak heights were not significantly different for hexane-extracted carinata presscake and carinata meal, while other total CHO structure spectral features were distinct ($P<0.05$). According to the multivariate analysis, carinata meal did not separate fully from canola meal in both CLA and PCA plots [Figure 4.5.2 (1), (2)], which indicated similar total CHO spectral characteristics between carinata meal and canola meal within the entire total CHO region. Similarities were found for the comparison between carinata meal and presscake, in accordance with mixed groups shown in CLA and PCA plots. However, the hexane-extracted carinata presscake and canola meal were partitioned into two clusters under a linkage distance of 1, and two individual ellipses with over 99% of the variation explained by PC1.

Carinata meal had a similar area ratio of STCHO to CELC with hexane-extracted carinata presscake (3.15 vs. 2.73), but a lower ratio compared with canola meal (5.09). Area ratios of

STCHO to total CHO and ratios of CELC to total CHO were higher for hexane-extracted carinata presscake but lower for carinata meal (0.29 vs. 0.27, 0.11 vs.0.09, respectively). To summarize, carinata meal, hexane-extracted carinata presscake and canola meal differed primarily from each other in the structural CHO and total CHO regions. In contrast, the cellulosic compounds spectral features were similar amongst three co-products according to the univariate analysis. The multivariate analyses revealed similarities of their carbohydrate structure spectral characteristics, while the hexane-extracted carinata presscake had distinct total CHO features to canola meal.

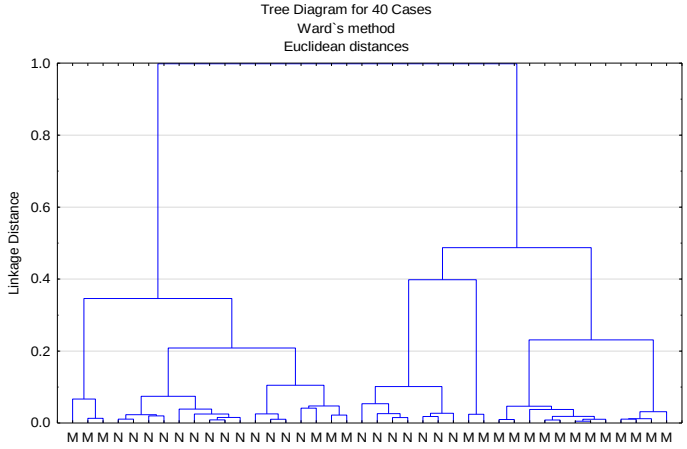
Table 4.3 Carbohydrate structure spectral characteristics of new carinata seeds (Yellow-AAC A110 vs. Brown-110915EM) in comparison with new canola seeds (Yellow-YN07 C1386 vs. Brown-N07 1374) and a commercial canola seed (Brown) using FTIR vibrational spectroscopy

Components	Peak region and center (cm^{-1})	New Carinata Seeds (N_CN)		New Canola Seeds (N_CL)		Commercial Canola Seed (COMM)	SEM	P value	Contrast, P value		
		Yellow (AAC A110)	Brown (110915 EM)	Yellow (YN07 C1386)	Brown (N07 1374)	Brown			N_CN vs N_CL	N_CN vs. COMM	Yellow vs Brown
Structural CHO (STCHO)											
STCHO peak 1 height	~1415	0.019^{b}	0.022^{a}	0.022^{a}	0.021^{ab}	0.020^{ab}	0.0007	0.01	0.10	0.43	0.23
STCHO peak 2 height	~1377	0.012^{c}	0.014^{b}	0.018^{a}	0.015^{b}	0.015^{b}	0.0004	<0.001	<0.001	<0.001	0.86
STCHO peak 3 height	~1234	0.023^{a}	0.023^{a}	0.018^{b}	0.017^{b}	0.017^{b}	0.0008	<0.001	<0.001	<0.001	0.75
STCHO area	1483-1193	3.58^{ab}	4.04^{a}	3.84^{ab}	3.69^{ab}	3.39^{b}	0.129	0.01	0.72	0.01	0.24
Cellulosic compounds (CELC)											
CELC peak height	~1234	0.021^{a}	0.021^{a}	0.015^{b}	0.014^{b}	0.015^{b}	0.0006	<0.001	<0.001	<0.001	0.53
CELC area	1304-1193	1.12^{a}	1.15^{a}	0.71^{b}	0.72^{b}	0.72^{b}	0.039	<0.001	<0.001	<0.001	0.60
Total CHO (CHO)											
CHO peak 1 height	~1141	0.039^{b}	0.038^{b}	0.043^{a}	0.037^{b}	0.043^{a}	0.0008	<0.001	0.06	<0.001	0.0002
CHO peak 2 height	~1100	0.065^{ab}	0.068^{a}	0.060^{b}	0.061^{b}	0.062^{ab}	0.0016	0.01	0.001	0.04	0.29
CHO peak 3 height	~1050	0.102^{ab}	0.109^{a}	0.111^{a}	0.106^{ab}	0.096^{b}	0.0031	0.01	0.28	0.02	0.72
CHO peak 1 area	1193-1128	1.56^{b}	1.57^{b}	1.76^{a}	1.56^{b}	1.73^{a}	0.037	<0.001	0.02	0.001	0.02
CHO peak 2 area	1128-1090	2.20	2.39	2.31	2.32	2.57	0.150	0.51	0.87	0.13	0.51
CHO peak 3 area	1090-883	9.68^{b}	10.55^{ab}	11.81^{a}	11.20^{a}	9.71^{b}	0.371	<0.001	0.001	0.37	0.73
Total CHO area	1193-883	13.44^{c}	14.51^{abc}	15.88^{a}	15.08^{ab}	14.02^{bc}	0.384	0.001	<0.001	0.93	0.72

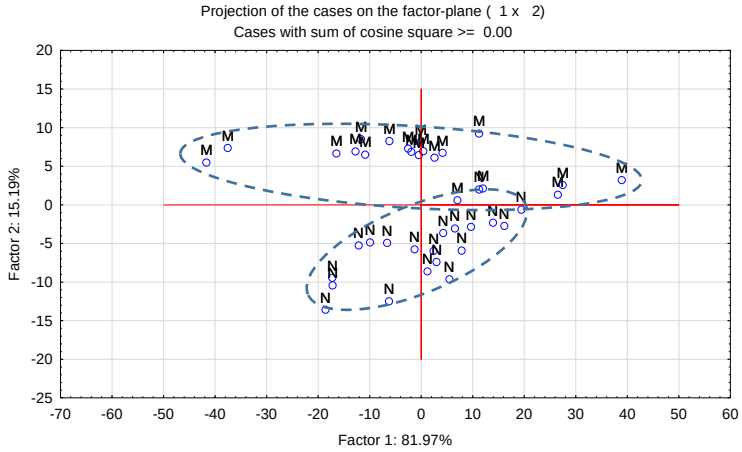
Table 4.3 Cont'd

Components	Peak region and center (cm^{-1})	New Carinata Seeds (N_CN)		New Canola Seeds (N_CL)		Commercial Canola Seed (COMM)	SEM	P value	Contrast, P value		
		Yellow (AAC A110)	Brown (110915 EM)	Yellow (YN07 C1386)	Brown (N07 1374)	Brown			N_CN vs N_CL	N_CN vs. COMM	Yellow vs Brown
Area ratio of STCHO to CELC		3.21[d]	3.55[c]	5.41[a]	5.11[a]	4.73[b]	0.078	<0.001	<0.001	<0.001	0.77
Area ratio of STCHO to CHO		0.27[a]	0.28[a]	0.24[b]	0.25[b]	0.24[b]	0.003	<0.001	<0.001	<0.001	0.09
Area ratio of CELC to CHO		0.08[a]	0.08[a]	0.05[b]	0.05[b]	0.05[b]	0.002	<0.001	<0.001	<0.001	1.00

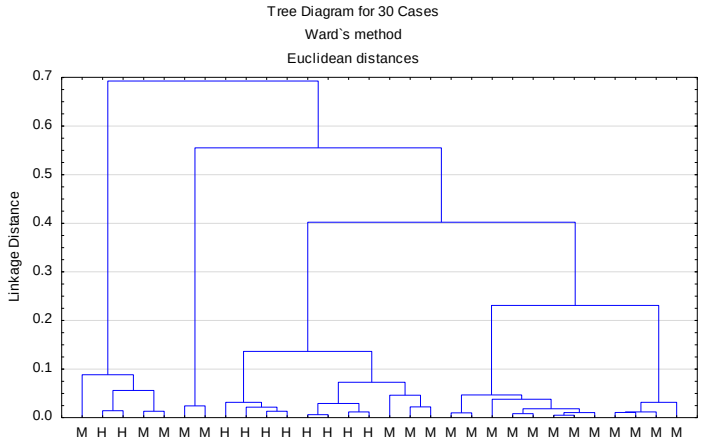
Notes: SEM: standard error of the mean. CHO, carbohydrate. Means with different superscripts in the same row are significantly different according to Tukey method ($P<0.05$).



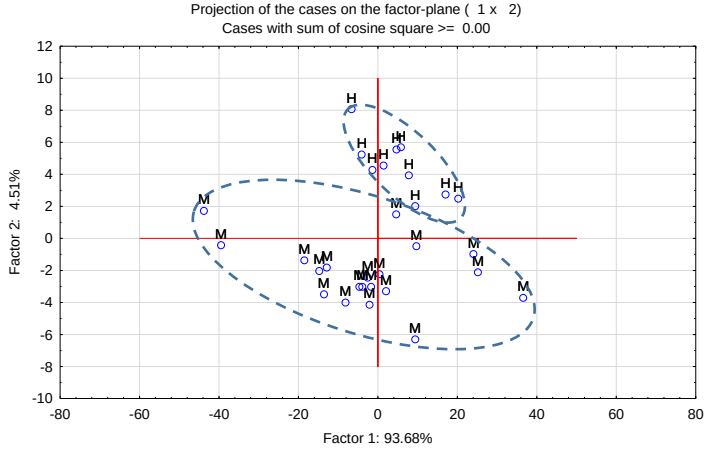
(1) CLA Comparison: Carinata Seeds (M) & Canola Seeds (N)



(2) PCA Comparison: Carinata Seeds (M) & Canola Seeds (N)

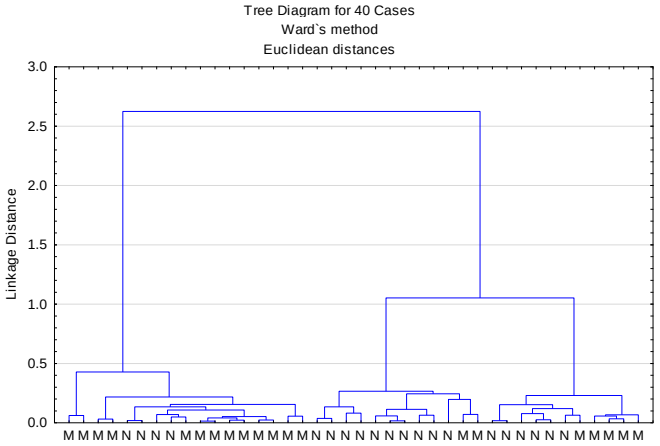


(3) CLA Comparison: Carinata Seeds (M) & Commercial Canola Seeds (H)

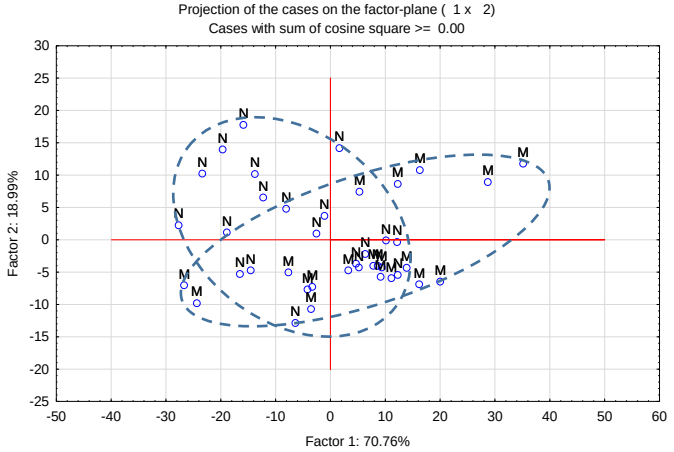


(4) PCA Comparison: Carinata Seeds (M) & Commercial Canola Seeds (H)

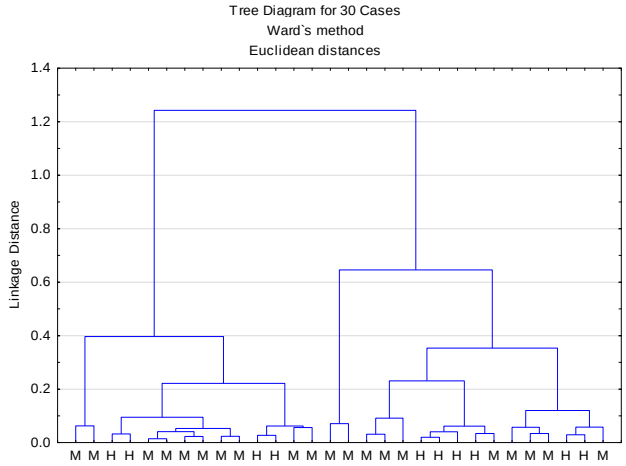
Figure 4.4.1 Multivariate spectral analyses of new carinata seeds (Yellow-AAC A110 and Brown-110915EM) in comparison with new canola seeds (Yellow-YN07 C1386 and Brown-N07 1374) and commercial canola seed (Brown) using FTIR vibrational spectroscopy at structural carbohydrate structure region (ca. 1483-1193 cm^{-1}). CLA (cluster analysis): cluster method (Ward's algorithm) and distance method (Euclidean). PCA (principal component analysis): Scatter plots of the 1st principal components (PC1) vs. the 2nd principal components (PC2)



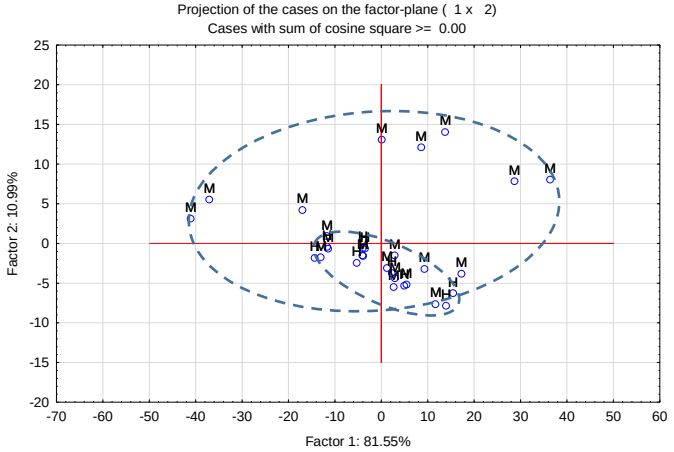
(1) CLA Comparison: Carinata Seeds (M) & Canola Seeds (N)



(2) PCA Comparison: Carinata Seeds (M) & Canola Seeds (N)



(3) CLA Comparison: Carinata Seeds (M) & Commercial Canola Seeds (H)



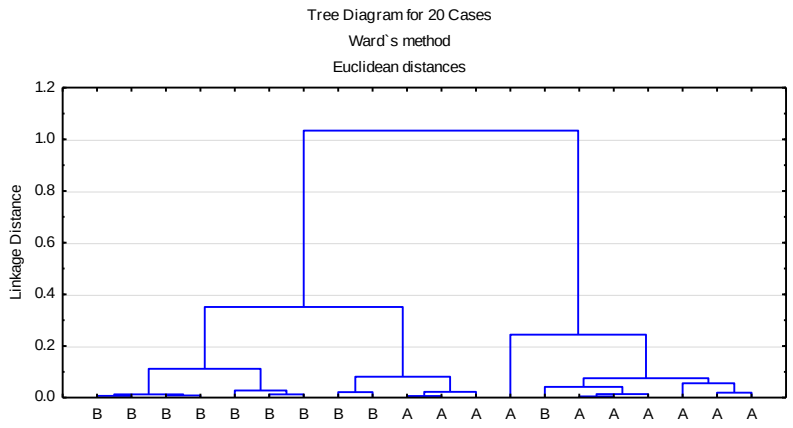
(4) PCA Comparison: Carinata Seeds (M) & Commercial Canola Seeds (H)

Figure 4.4.2 Multivariate spectral analyses of new carinata seeds (Yellow-AAC A110 and Brown-110915EM) in comparison with new canola seeds (Yellow-YN07 C1386 and Brown-N07 1374) and commercial canola seed (Brown) using FTIR vibrational spectroscopy at total carbohydrate structure region (ca. 1193-883 cm^{-1}). CLA (cluster analysis): cluster method (Ward's algorithm) and distance method (Euclidean). PCA (principal component analysis): Scatter plots of the 1st principal components (PC1) vs. the 2nd principal components (PC2)

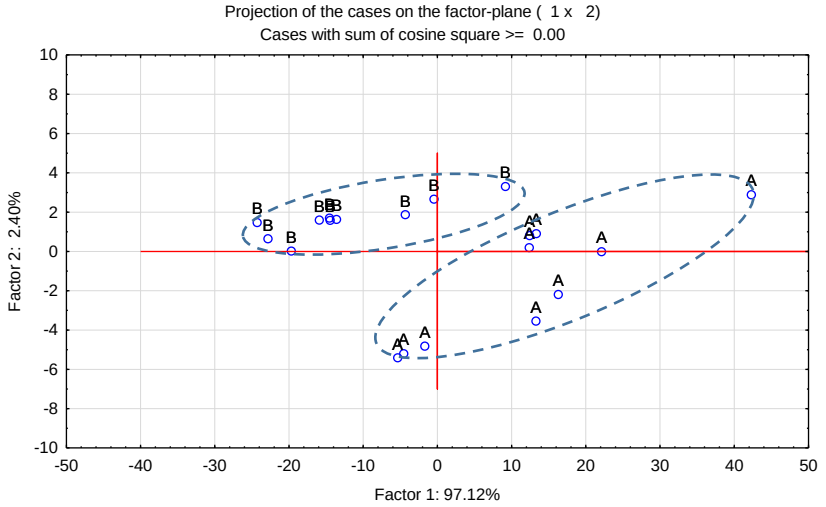
Table 4.4 Carbohydrate structure spectral characteristics of carinata meal and hexane-extracted carinata presscake in comparison with canola meal using FTIR vibrational spectroscopy

Components	Peak region and center (cm^{-1})	Carinata Meal	Carinata Presscake	Canola Meal	SEM	P value
Structural CHO (STCHO)						
STCHO peak 1 height	~1415	0.011^{b}	0.008^{c}	0.017^{a}	0.0005	<0.001
STCHO peak 2 height	~1373	0.005^{b}	0.004^{c}	0.011^{a}	0.0003	<0.001
STCHO peak 3 height	~1234	0.012	0.011	0.012	0.0005	0.53
STCHO area	1479-1182	1.96^{b}	1.65^{c}	2.77^{a}	0.086	<0.001
Cellulosic compounds (CELC)						
CELC peak height	~1234	0.011	0.010	0.011	0.0005	0.87
CELC area	1307-1182	0.63	0.61	0.55	0.030	0.22
Total CHO (CHO)						
CHO peak 1 height	~1154	0.014^{b}	0.010^{c}	0.022^{a}	0.0007	<0.001
CHO peak 2 height	~1105	0.034^{b}	0.025^{c}	0.047^{a}	0.0015	<0.001
CHO peak 3 height	~1051	0.052^{b}	0.045^{b}	0.074^{a}	0.0025	<0.001
CHO peak 1 area	1192-1130	0.66^{b}	0.52^{c}	0.93^{a}	0.033	<0.001
CHO peak 2 area	1130-1090	1.25^{b}	0.85^{c}	1.79^{a}	0.056	<0.001
CHO peak 3 area	1090-881	5.32^{b}	4.36^{c}	8.12^{a}	0.244	<0.001
Total CHO area	1192-881	7.24^{b}	5.73^{c}	10.84^{a}	0.324	<0.001
Area ratio of STCHO to CELC		3.15^{b}	2.73^{b}	5.09^{a}	0.156	<0.001
Area ratio of STCHO to CHO		0.27^{b}	0.29^{a}	0.26^{c}	0.003	<0.001
Area ratio of CELC to CHO		0.09^{b}	0.11^{a}	0.05^{c}	0.003	<0.001

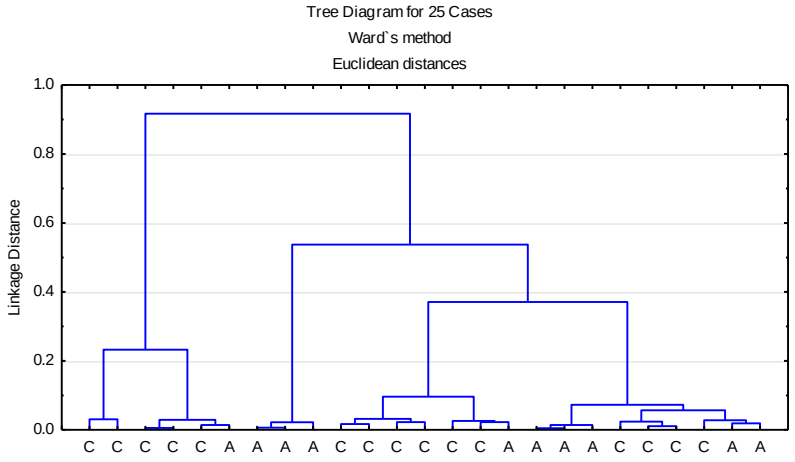
Notes: SEM: standard error of the mean. CHO, carbohydrate. Means with different superscripts in the same row are significantly different according to Tukey method ($P<0.05$).



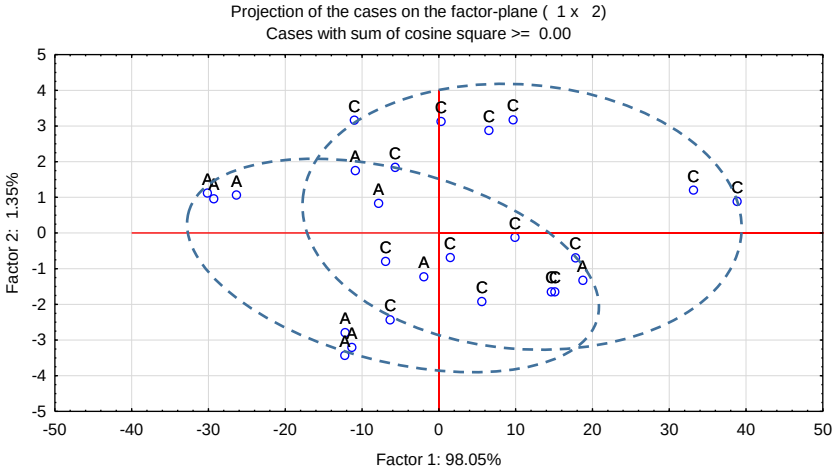
(1) CLA Comparison: Carinata Meal (A) and Canola Meal (B)



(2) PCA Comparison: Carinata Meal (A) and Canola Meal (B)

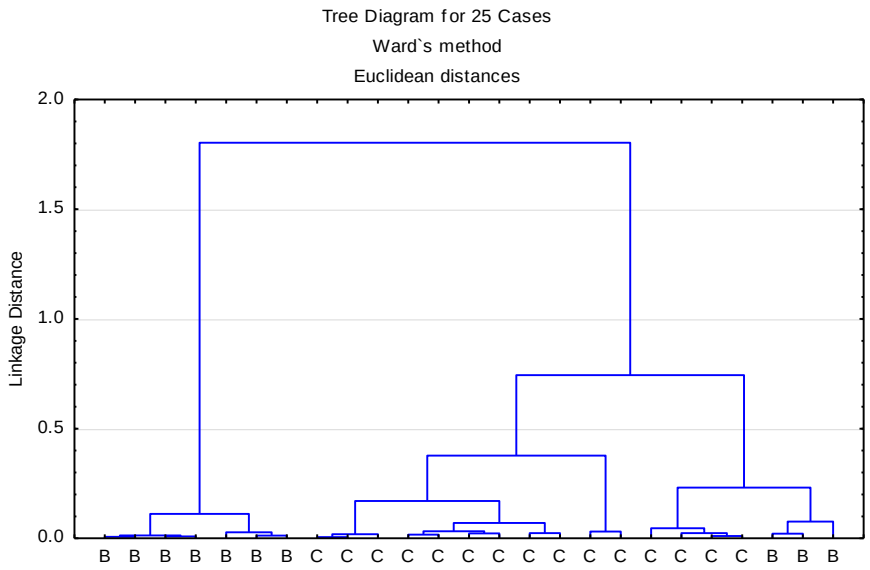


(3) CLA Comparison: Carinata Meal (A) and Carinata Presscake (C)

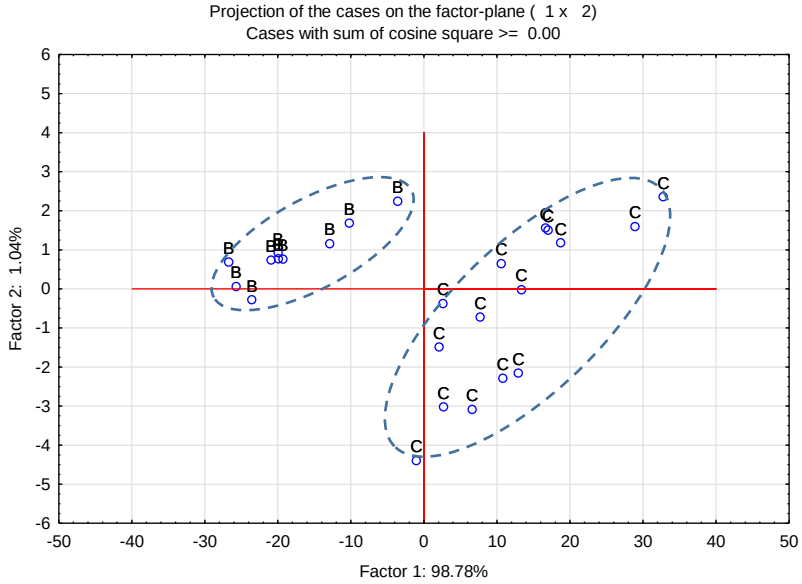


(4) PCA Comparison: Carinata Meal (A) and Carinata Presscake (C)

Figure 4.5.1 Multivariate spectral analyses of carinata meal (A) and hexane-extracted carinata presscake (C) in comparison with canola meal (B) using FTIR vibrational spectroscopy at structural carbohydrate structure region (ca. 1479-1182 cm^{-1}). CLA (cluster analysis): cluster method (Ward's algorithm) and distance method (Euclidean). PCA (principal component analysis): Scatter plots of the 1st principal components (PC1) vs. the 2nd principal components (PC2)

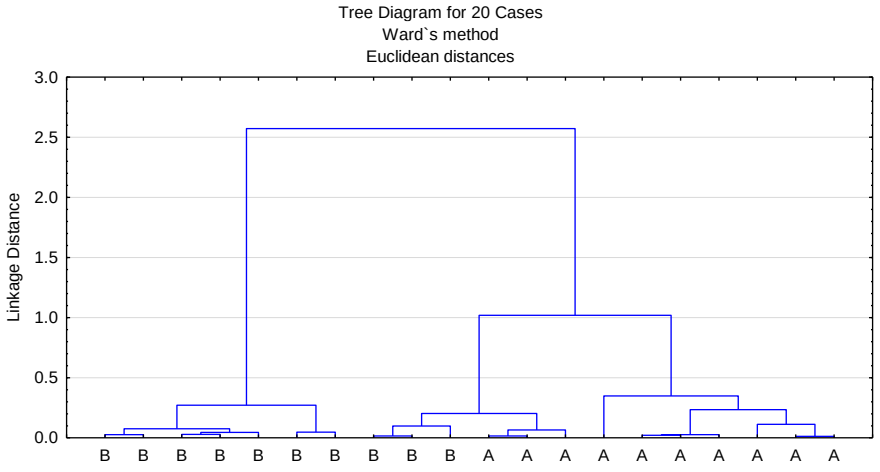


(5) CLA Comparison: Canola Meal (B) and Carinata Presscake (C)

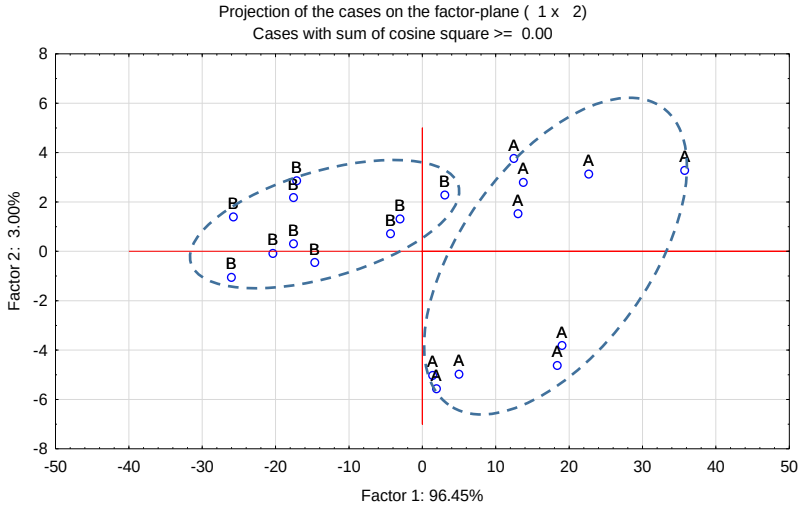


(6) PCA Comparison: Canola Meal (B) and Carinata Presscake (C)

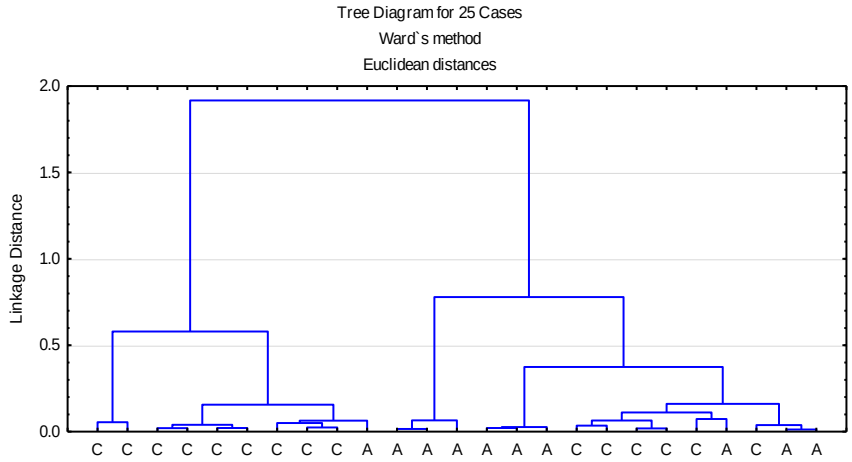
Figure 4.5.1 Cont'd



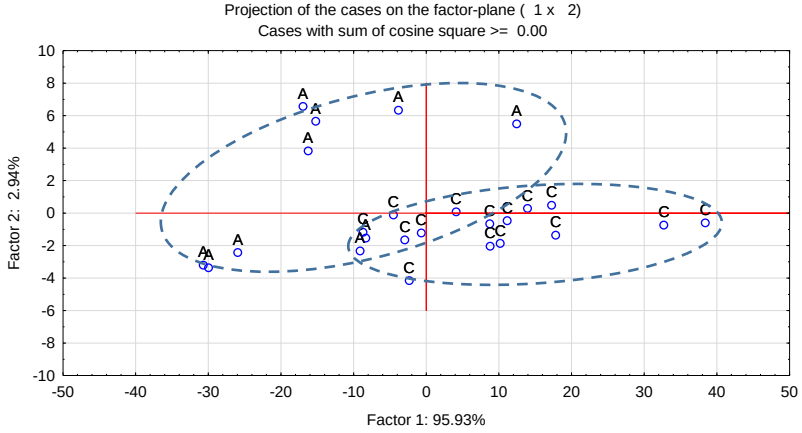
(1) CLA Comparison: Carinata Meal (A) and Canola Meal (B)



(2) PCA Comparison: Carinata Meal (A) and Canola Meal (B)

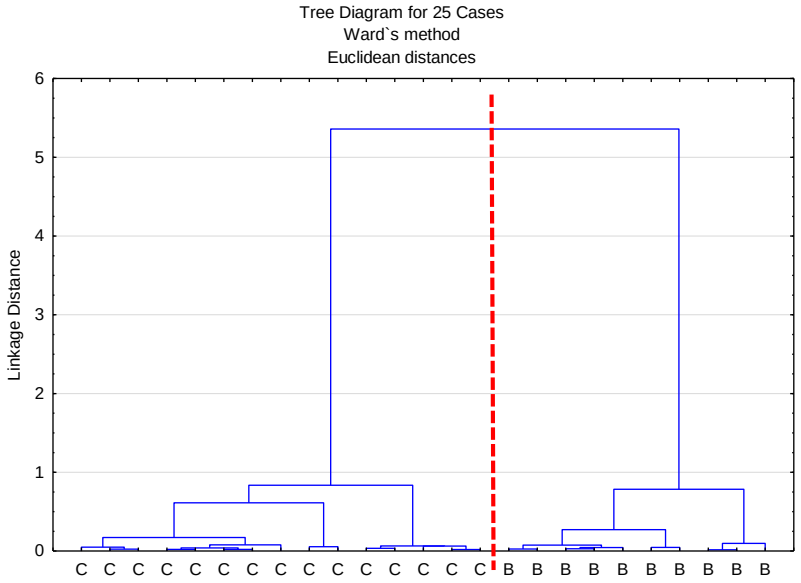


(3) CLA Comparison: Carinata Meal (A) and Carinata Presscake (C)

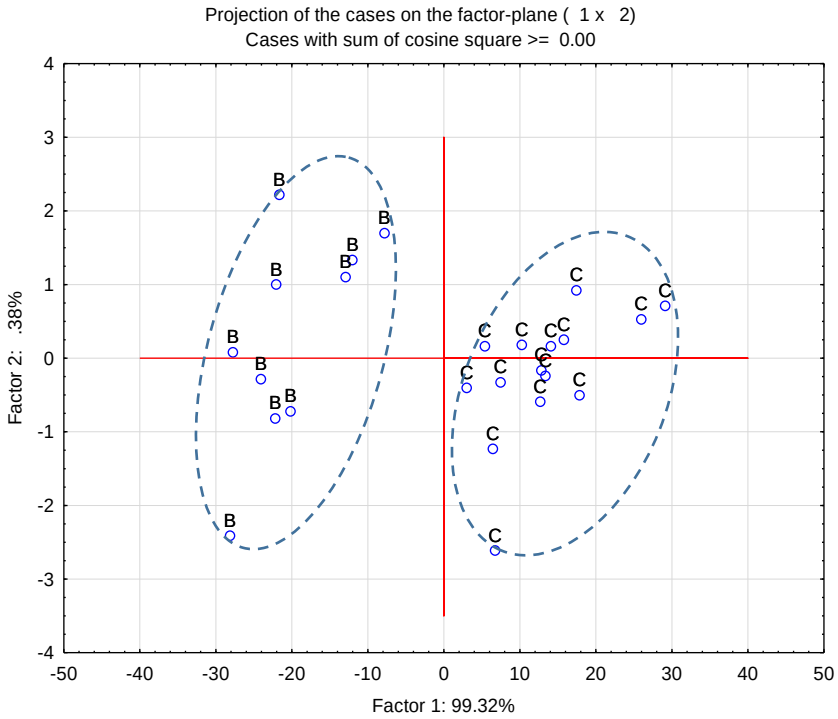


(4) PCA Comparison: Carinata Meal (A) and Carinata Presscake (C)

Figure 4.5.2 Multivariate spectral analyses of carinata meal (A) and hexane-extracted carinata presscake (C) in comparison with canola meal (B) using FTIR vibrational spectroscopy at total carbohydrate structure region (ca. 1192-881 cm^{-1}). CLA (cluster analysis): cluster method (Ward's algorithm) and distance method (Euclidean). PCA (principal component analysis): Scatter plots of the 1st principal components (PC1) vs. the 2nd principal components (PC2)



(5) CLA Comparison: Canola Meal (B) and Carinata Presscake (C)



(6) PCA Comparison: Canola Meal (B) and Carinata Presscake (C)

Figure 4.5.2 Cont'd

4.3.3. Correlation Study between Nutrient Structural Spectral Features and Bioavailability

4.3.3.1. Correlation Study between Nutrient Structural Spectral Features and Bioavailability in Carinata and Canola Seeds

4.3.3.1.1. Correlation Study between Protein Structure Spectral Features and Protein Profiles, Rumen Degradation and Intestinal Digestion Characteristics, and Predicted Truly Absorbed Protein Supply

Carinata seeds showed different nutritional values and protein availability compared with canola seeds, which may be partially due to the differences in their protein internal molecular structure. In this case, a correlation study was conducted to reveal the relationship between protein structural features and protein bioavailability (Table 4.5). Crude protein tended to have a positive correlation with amide I peak height, area and amide II peak height ($P<0.10$). NDICP was negatively correlated to amide I peak height ($r=-0.72$, $P<0.05$), area ($r=-0.68$, $P<0.05$), amide II peak height ($r=-0.71$, $P<0.05$), α-helix height ($r=-0.77$, $P<0.01$) and height ratio of α-helix to β-sheet ($r=-0.71$, $P<0.05$). However, only height ratio of α-helix to β-sheet showed a negative correlation to ADICP of carinata seeds and canola seeds ($r=-0.67$, $P<0.05$). Area ratio of amide I to amide II was negatively correlated to SCP ($r=-0.67$, $P<0.05$), while height ratio of amide I to amide II showed a tendency of negative correlation ($r=-0.61$, $P<0.10$). Non-protein nitrogen (NPN) was positively correlated to amide I peak height ($r=0.66$, $P<0.05$), α-helix height ($r=0.73$, $P<0.05$) as well as height ratio of α-helix to β-sheet ($r=0.71$, $P<0.05$), whereas amide I area and amide II peak height tended to have a positive correlation ($P<0.10$).

Protein structural parameters of carinata seeds and canola seeds in our study were positively correlated to ruminal protein degradation features. Rumen undegraded protein (bypass protein) was significantly positively influenced by amide I peak height and area, amide II peak height and area, and protein secondary structure ($P<0.05$), indicating that higher absorption intensities of protein-relative functional groups contributed to higher RUP. EDCP tended to be

positively correlated with amide I peak height and area as well as height ratio of α-helix to β-sheet ($P<0.10$). No correlation was observed between intestinal protein digestion and protein structure ($P\geq0.10$), though a higher height ratio of α-helix to β-sheet would result in more TDP ($P<0.05$). Based on the Dutch DVE/OEB model, height ratio of α-helix to β-sheet had a positive correlation with DVE value ($r=0.64$, $P<0.05$), which equally correlated to feed milk value. OEB was observed positively correlated to amide I peak height, area and amide II peak height ($P<0.05$).

Yu (2005b) found that β-sheet height had a negative correlation to protein digestion; our study proved that, with a positive correlation observed between β-sheet height and rumen bypass protein (BCP^{DVE}), which indicated a negative correlation between β-sheet height and rumen protein digestion. This difference may due to different feed types and processing. As previously stated, spectral characteristics of protein structure showed positive influences on rumen undegraded protein, total digestible protein and truly digested protein in the small intestine for carinata seeds and canola seeds in this study.

4.3.3.1.2. Correlation Study between Carbohydrate Structure Spectral Features and Carbohydrate Profiles, Rumen Degradation and Intestinal Digestion Characteristics, and Predicted Truly Absorbed Protein Supply

Protein biodegradation characteristics are not only correlated with protein structure of feed, but also with carbohydrate structure features (Xin et al., 2013c, d). Peng et al. (2014b) also found a relationship between carbohydrate molecular structure and rumen degradation of protein. Thus, carbohydrate spectral profiles should be included in the correlation study of molecular structure and nutritive digestion characteristics.

In our study, carbohydrate spectral characteristics of carinata and canola seeds showed positive correlation with protein digestion (Table 4.6). For rumen degradation, BCP was positively correlated to structural CHO 3rd peak height ($r=0.67$, $P<0.05$), cellulosic compounds peak height

(r=0.64, P<0.05) and area (r=0.63, P<0.05). Moreover, a higher effectively degraded CP would be associated with a higher STCHO 3rd peak height (r=0.87, P<0.01), CELC peak height (r=0.78, P<0.01), area (r=0.78, P<0.01) or total CHO 2nd peak height (r=0.73, P<0.05), but a lower total CHO 1st peak height (r=-0.67, P<0.05). Intestinal digested protein was positively associated with structural CHO area (r=0.64, P<0.05), while the total digested protein was positively altered by STCHO 3rd peak height (r=0.88, P<0.01), CELC peak height (r=0.81, P<0.01), area (r=0.79, P<0.01) and total CHO 2nd peak height (0.64, P<0.05). Carbohydrate spectral profiles were not significantly correlated to total DVE value and feed milk value; on the other hand, OEB value was positively correlated to STCHO 3rd peak height (r=0.84, P<0.01), CELC peak height (r=0.81, P<0.01), area (r=0.83, P<0.01) and total CHO 2nd peak height (r=0.87, P<0.01).

4.3.3.2. Correlation Study between Nutrient Structural Spectral Features and Bioavailability in Carinata and Canola Co-products

4.3.3.2.1. Correlation Study between Protein Structure Spectral Features and Protein Profiles, Rumen Degradation and Intestinal Digestion Characteristics, and Predicted Truly Absorbed Protein Supply

Area ratio of amide I to amide II was observed as negatively correlated to CP (r=-0.95, P<0.01), SCP (r=-0.83, P<0.05) and NPN (r=-0.86, P<0.05), positively associated with NDICP and ADICP (P<0.05) for carinata meal, hexane-extracted carinata presscake and canola meal, as shown in Table 4.7. Additionally, height ratio of α-helix to β-sheet was positively related to NPN (r=0.79, P<0.05). Huang (2015) reported a similar negative correlation between crude protein of various canola meal pellet with different pelleting conditions (different temperatures and processing time) and their area ratio of amide I to amide II, as well as a relationship between SCP and amide I peak height or height ratio of amide I to amide II (P<0.05). Rumen undegraded protein based on the DVE/OEB system was negatively correlated with height ratio of amide I to amide II (r=-0.77, P<0.05) and positively with area ratio of amide I to amide II (r=0.76, P<0.05), although

only area ratio of amide I to amide II had a negative correlation with EDCP (r=-0.83, P<0.05). Conversely, it was reported that protein secondary structure had positive relationships with in situ protein degradation kinetics (Kd, S and EDCP) for canola meal and canola presscake (Theodoridou and Yu, 2013a). A high value of intestinal digested protein may result from a lower height ratio of amide I to amide II (r=-0.77, P<0.05) and a higher area ratio (r=0.76, P<0.05). Nevertheless, the area ratio negatively affected total digested protein of the three co-products (r=-0.92, P<0.01). Theodoridou and Yu (2013a) also observed that protein secondary structure was significantly correlated with truly absorbed rumen synthesized microbial protein in the small intestine ($AMCP^{DVE}$) and truly absorbed protein in the small intestine (DVE). In contrast, for our study, DVE was correlated with all protein structural profiles (P<0.05), except the area ratio of amide I to amide II and height ratio of α-helix to β-sheet. However, the area ratio of amide I to amide II was negatively correlated with degraded protein balance (OEB) (r=-0.83, P<0.05). In summary, for carinata meal, hexane-extracted carinata presscake and canola meal, area ratio of amide I to amide II showed correlations with protein profiles, rumen degradation and intestinal digestion of protein, as well as degraded protein balance value. In addition, truly digestible protein in the small intestine, based on the DVE/OEB system, was positively related to most of the protein structural parameters.

4.3.3.2.2. Correlation Study between Carbohydrate Structure Spectral Features and Carbohydrate Profiles, Rumen Degradation and Intestinal Digestion Characteristics, and Predicted Truly Absorbed Protein Supply

Table 4.8 shows the correlation analyses between CHO structure of carinata meal, carinata presscake and canola meal and nutritive bioavailability. Structural CHO 2^{nd} peak height was correlated to carbohydrate for the three co-products (r=0.79, P<0.05). NDF was positively influenced by STCHO 1^{st} and 2^{nd} peak heights, total area, total CHO 1^{st} and 3^{rd} peak areas, while

ADF and ADL had correlations to STCHO 1st and 2nd peak heights, total area, total CHO 1st and 2nd peak heights, 2nd and 3rd peak areas as well as total CHO area ($P<0.05$). Our results showed that rumen degradable protein was negatively correlated to some carbohydrate structural profiles but rumen bypass protein was positively associated. Therefore, a higher spectral absorption intensity at CHO region may indicate a lower ruminal protein degradation but a higher intestinal protein availability for carinata meal or hexane-extracted carinata presscake. Related with RUP, intestinal digested protein was similarly positively correlated with STCHO 1st and 2nd peak heights, total area, total CHO peak heights and area ($P<0.05$). As for total digestible protein and CHO structure, a negative correlation was found. Furthermore, strong positive correlation was observed from the analysis between DVE value and CHO structure ($P<0.01$), and different from results for carinata and canola seeds as explained above. OEB value was negatively linked with STCHO 1st peak height ($r=-0.93$, $P<0.01$), 2nd peak height ($r=-0.96$, $P<0.01$), total area ($r=-0.93$, $P<0.01$), total CHO 1st and 2nd peak heights as well as peak areas ($r=-0.86$ and $P<0.05$ for each), 3rd peak area ($r=-0.93$, $P<0.01$) and total CHO area ($r=-0.86$, $P<0.05$).

Therefore, given the correlation among them, the differences in protein and carbohydrate structural features should relate to distinct nutrient bioavailability in carinata meal, hexane-extracted carinata presscake and canola meal.

Table 4.5 Correlation analyses between protein structure spectral characteristics and protein profiles, rumen protein degradation, intestinal protein digestion and predicted truly absorbed protein supply of new carinata seeds (Yellow-AAC A110 and Brown-110915EM), new canola seeds (Yellow-YN07 C1386 and Brown-N07 1374) and a commercial canola seed (Brown)

Items	Amide I height	Amide I area	Amide II height	Amide II area	Height ratio of amide I to amide II	Area ratio of amide I to amide II	α-helix height	β-sheet height	Height ratio of α-helix to β-sheet
	----------Spearman Correlation R values----------								
Protein profiles									
CP (%DM)	0.62^{+}	0.61^{+}	0.55^{+}	0.50	0.29	-0.29	0.53	0.48	0.49
NDICP (%CP)	-0.72*	-0.68*	-0.71*	-0.54	-0.37	-0.18	-0.77**	-0.49	-0.71*
ADICP (%CP)	-0.37	-0.36	-0.33	-0.16	-0.50	-0.44	-0.39	-0.12	-0.67*
SCP (%CP)	-0.05	-0.03	0.04	-0.03	-0.61^{+}	-0.67*	0.01	0.07	-0.38
NPN (%CP)	0.66*	0.56^{+}	0.60^{+}	0.45	0.28	-0.26	0.73*	0.43	0.71*
Rumen protein degradation									
BCP^{DVE} (g/kg DM)	0.74*	0.75*	0.71*	0.68*	0.07	-0.43	0.74*	0.73*	0.29
EDCP (g/kg DM)	0.59^{+}	0.58^{+}	0.53	0.45	0.35	-0.18	0.49	0.41	0.58^{+}
Intestinal protein digestion									
IDP (g/kg DM)	0.24	0.22	0.19	0.24	0.01	0.17	0.31	0.22	0.40
TDP (g/kg DM)	0.63^{+}	0.60^{+}	0.55^{+}	0.45	0.48	0.10	0.58^{+}	0.39	0.72*
DVE/OEB model									
DVE (g/kg DM)	0.46	0.42	0.39	0.35	0.15	0.14	0.53	0.32	0.64*
OEB (g/kg DM)	0.71*	0.71*	0.67*	0.62^{+}	0.36	-0.30	0.58^{+}	0.58^{+}	0.38
FMV (kg milk/kg feed)	0.46	0.42	0.39	0.35	0.15	0.14	0.53	0.32	0.64*

Notes: R: correlation coefficient calculated using Spearman method. DM: dry matter; CP: crude protein; NDICP: neutral detergent insoluble crude protein; ADICP: acid detergent insoluble crude protein; SCP: soluble crude protein; NPN: non-protein nitrogen; BCP^{DVE}: rumen bypassed crude protein in DVE/OEB system; EDCP: effectively degraded crude protein; IDP: intestinal digested protein; TDP: total digested protein; DVE: truly digested protein in the small intestine; OEB: degraded protein balance; FMV^{DVE}: feed milk value in DVE/OEB system. "+": P<0.10; "*": P<0.05; "**": P<0.01.

Table 4.6 Correlation analyses between carbohydrate structure spectral characteristics and carbohydrate profiles, rumen protein degradation, intestinal protein digestion and predicted truly absorbed protein supply of new carinata seeds (Yellow-AAC A110 and Brown-110915EM), new canola seeds (Yellow-YN07 C1386 and Brown-N07 1374) and a commercial canola seed (Brown)

Items	STCHO peak 1 height	STCHO peak 2 height	STCHO peak 3 height	STCHO area	CELC height	CELC area	Total CHO peak 1 height	Total CHO peak 2 height	Total CHO peak 3 height	Total CHO peak 1 area	Total CHO peak 2 area	Total CHO peak 3 area	Total CHO area
	----------Spearman Correlation R values----------												
CHO profiles													
CHO (%DM)	0.45	0.15	-0.13	0.21	-0.16	-0.16	-0.13	0.01	0.26	-0.05	0.24	0.26	0.31
NDF (%DM)	0.22	0.21	-0.35	-0.05	-0.34	-0.31	-0.25	-0.07	0.07	-0.21	0.05	0.33	0.32
ADF (%DM)	0.18	0.28	-0.48	-0.21	-0.47	-0.43	-0.05	-0.13	-0.15	-0.05	0.15	0.16	0.30
ADL (%DM)	0.16	0.27	-0.49	-0.25	-0.47	-0.43	-0.01	-0.16	-0.20	-0.02	0.13	0.13	0.27
Rumen protein degradation													
BCP^{DVE} (g/kg DM)	-0.05	-0.34	0.67*	0.36	0.64*	0.63*	-0.42	0.54	0.41	-0.22	-0.09	0.13	-0.14
EDCP (g/kg DM)	-0.17	-0.61^{+}	0.87**	0.31	0.78**	0.78**	-0.67*	0.73*	0.27	-0.58^{+}	-0.14	-0.27	-0.47
Intestinal protein digestion													
IDP (g/kg DM)	0.51	0.22	0.35	0.64*	0.35	0.30	0.13	0.15	0.56^{+}	0.25	0.35	0.42	0.27
TDP (g/kg DM)	-0.05	-0.46	0.88**	0.48	0.81**	0.79**	-0.50	0.64*	0.43	-0.42	-0.10	-0.10	-0.35
DVE/OEB system													
DVE (g/kg DM)	0.20	-0.10	0.59^{+}	0.54	0.57^{+}	0.53	-0.04	0.26	0.47	0.04	0.07	0.20	-0.05
OEB (g/kg DM)	-0.21	-0.63^{+}	0.84**	0.22	0.81**	0.83**	-0.61^{+}	0.87**	0.14	-0.49	-0.03	-0.43	-0.53
FMV (kg milk/kg feed)	0.20	-0.10	0.59^{+}	0.54	0.57^{+}	0.53	-0.04	0.26	0.47	0.04	0.07	0.20	-0.05

Notes: R: correlation coefficient calculated using Spearman method. DM: dry matter; CHO: carbohydrate; STCHO: structural CHO; CELC: cellulosic compounds; NDF: neutral detergent fiber; ADF: acid detergent fiber; ADL: acid detergent lignin; BCP^{DVE}: rumen bypass crude protein in DVE/OEB system; EDCP: effectively degraded crude protein; IDP: intestinal digested protein; TDP: total digested protein; DVE: truly digested protein in the small intestine; OEB: degraded protein balance; FMV^{DVE}: feed milk value in DVE/OEB systeml. "+": P<0.10; "*": P<0.05; "**": P<0.01.

Table 4.7 Correlation analyses between protein structure spectral characteristics and protein profiles, rumen protein degradation, intestinal protein digestion and predicted truly absorbed protein supply of carinata meal, hexane-extracted carinata presscake and canola meal

Items	Amide I height	Amide I area	Amide II height	Amide II area	Height ratio of amide I to amide II	Area ratio of amide I to amide II	α-helix height	β-sheet height	Height ratio of α-helix to β-sheet
	----------Spearman Correlation R values----------								
Protein profiles									
CP (%DM)	-0.14	-0.32	-0.32	-0.14	0.49	-0.95**	-0.14	-0.32	0.50
NDICP (%CP)	0.54	0.64	0.64	0.54	-0.63	0.83*	0.54	0.64	-0.36
ADICP (%CP)	0.25	0.36	0.36	0.25	-0.47	0.85*	0.25	0.36	-0.36
SCP (%CP)	-0.54	-0.64	-0.64	-0.54	0.63	-0.83*	-0.54	-0.64	0.36
NPN (%CP)	0.07	-0.14	-0.14	0.07	0.40	-0.86*	0.07	-0.14	0.79*
Rumen protein degradation									
BCP^{DVE} (g/kg DM)	0.64	0.75^{+}	0.75^{+}	0.64	-0.77*	0.76*	0.64	0.75^{+}	-0.39
EDCP (g/kg DM)	-0.54	-0.64	-0.64	-0.54	0.63	-0.83*	-0.54	-0.64	0.36
Intestinal protein digestion									
IDP (g/kg DM)	0.64	0.75^{+}	0.75^{+}	0.64	-0.77*	0.76*	0.64	0.75^{+}	-0.39
TDP (g/kg DM)	-0.36	-0.50	-0.50	-0.36	0.58	-0.92**	-0.36	-0.50	0.46
DVE/OEB system									
DVE (g/kg DM)	0.79*	0.86*	0.86*	0.79*	-0.77*	0.67	0.79*	0.86*	-0.43
OEB (g/kg DM)	-0.54	-0.64	-0.64	-0.54	0.63	-0.83*	-0.54	-0.64	0.36
FMV (kg milk/kg feed)	0.79*	0.86*	0.86*	0.79*	-0.77*	0.67	0.79*	0.86*	-0.43

Notes: R: correlation coefficient calculated using Spearman method. DM: dry matter; CP: crude protein; NDICP: neutral detergent insoluble crude protein; ADICP: acid detergent insoluble crude protein; SCP: soluble crude protein; NPN: non-protein nitrogen; BCP^{DVE}: rumen bypass crude protein in DVE/OEB system; EDCP: effectively degraded crude protein; IDP: intestinal digested protein; TDP: total digested protein; DVE: truly digested protein in the small intestine; OEB: degraded protein balance; FMV^{DVE}: feed milk value in DVE/OEB system. "+": $P<0.10$; "*": $P<0.05$; "**": $P<0.01$.

Table 4.8 Correlation analyses between carbohydrate structure spectral characteristics and carbohydrate profiles, rumen protein degradation, intestinal protein digestion and predicted truly absorbed protein supply of carinata meal, hexane-extracted carinata presscake and canola meal

Items	STCHO peak 1 height	STCHO peak 2 height	STCHO peak 3 height	STCHO area	CELC height	CELC area	Total CHO peak 1 height	Total CHO peak 2 height	Total CHO peak 3 height	Total CHO peak 1 area	Total CHO peak 2 area	Total CHO peak 3 area	Total CHO area
	----------Spearman Correlation R values----------												
CHO profiles													
CHO (%DM)	0.71^{+}	0.79*	-0.29	0.71^{+}	-0.29	-0.71^{+}	0.61	0.61	0.46	0.68	0.61^{+}	0.71^{+}	0.61^{+}
NDF (%DM)	0.86*	0.89**	-0.07	0.86*	-0.07	-0.57	0.75^{+}	0.75^{+}	0.61	0.82*	0.75^{+}	0.86*	0.75^{+}
ADF (%DM)	0.86*	0.93**	0.00	0.86*	0.00	-0.64	0.82*	0.82*	0.68^{+}	0.75^{+}	0.82*	0.86*	0.82*
ADL (%DM)	0.79*	0.82*	0.07	0.79*	0.07	-0.64	0.82*	0.82*	0.71^{+}	0.64	0.82*	0.79*	0.82*
Rumen protein degradation													
BCP^{DVE} (g/kg DM)	0.96**	0.93**	0.25	0.96**	0.25	-0.36	0.93**	0.93**	0.82*	0.89**	0.93**	0.96**	0.93**
EDCP (g/kg DM)	-0.93**	-0.96**	-0.07	-0.93**	-0.07	0.54	-0.86*	-0.86*	-0.71^{+}	-0.86*	-0.86*	-0.93**	-0.86*
Intestinal protein digestion													
IDP (g/kg DM)	0.96**	0.93**	0.25	0.96**	0.25	-0.36	0.93**	0.93**	0.82*	0.89**	0.93**	0.96**	0.93**
TDP (g/kg DM)	-0.86*	-0.89**	0.07	-0.86*	0.07	0.57	-0.75^{+}	-0.75^{+}	-0.61	-0.82*	-0.75^{+}	-0.86*	-0.75^{+}
DVE/OEB system													
DVE (g/kg DM)	1.00**	0.96**	0.43	1.00**	0.43	-0.29	0.96**	0.96**	0.89**	0.96**	0.96**	1.00**	0.96**
OEB (g/kg DM)	-0.93**	-0.96**	-0.07	-0.93**	-0.07	0.54	-0.86*	-0.86*	-0.71^{+}	-0.86*	-0.86*	-0.93**	-0.86*
FMV (kg milk/kg feed)	1.00**	0.96**	0.43	1.00**	0.43	-0.29	0.96**	0.96**	0.89**	0.96**	0.96**	1.00**	0.96**

Notes: R: correlation coefficient calculated using Spearman method. DM: dry matter; CHO: carbohydrate; STCHO: structural CHO; CELC: cellulosic compounds; NDF: neutral detergent fiber; ADF: acid detergent fiber; ADL: acid detergent lignin; BCP^{DVE}: rumen bypass crude protein in DVE/OEB system; EDCP: effectively degraded crude protein; IDP: intestinal digested protein; TDP: total digested protein; DVE: truly digested protein in the small intestine; OEB: degraded protein balance; FMV^{DVE}: feed milk value in DVE/OEB system. "+": P<0.10; "*": P<0.05; "**": P<0.01.

4.3.4. Regression Study of Nutrient Structural Spectral Features and Bioavailability

4.3.4.1. Regression Study of Nutrient Structural Spectral Features and Bioavailability in Carinata and Canola Seeds

4.3.4.1.1. Regression Study of Protein Structure Spectral Features and Protein Profiles, Rumen Degradation and Intestinal Digestion Characteristics, and Predicted Truly Absorbed Protein Supply

Given the inherent relationships of protein structure and protein availability, multiple regression analyses were conducted to select variables to predict protein profiles, ruminal degradation kinetics, intestinal digestion and truly absorbed protein supply for dairy cattle (Table 4.9). The conventional animal trial is time consuming and expensive, then it is essential to develop a fast prediction method to predict nutrient bioavailability of feeds for animals. The tested multiple regression model was Y = amide I peak height (H_AI) + amide I area (A_AI) + amide II peak height (H_AII) + amide II area (A_AII) + height ratio of amide I to amide II (H_AI_II) + area ratio of amide I to amide II (A_AI_II) + α-helix height (H_A) + β-sheet height (H_B) + height ratio of α-helix to β-sheet (H_AB), with variables ($P<0.05$) selected to leave in the prediction equation. A higher percentage of the total variance represents a higher variance being explained in the model.

The height of α-helix was left in the model as the only predictor for CP, NDICP and NPN of carinata seeds and canola seeds, which accounted for 56%, 56% and 52% of the total variance, respectively. As for rumen protein degradation, the amide II area could be used to predict BCP^{DVE} with 68% of the variance reflected. Effectively degraded CP was predicted by amide I peak height and area, which represented 76% of the variance, where the same predictors could be used for degraded protein balance in accordance with DVE/OEB system. Total digested protein had the single predictor, α-helix height, accounting for 55% of the total variance. In summary, protein structural variables, such as amide I peak height, area, amide II area and α-helix height, could be

used as predictors for protein profiles as well as digestion characteristics for carinata seeds and canola seeds in our study.

4.3.4.1.2. Regression Study of Carbohydrate Structure Spectral Features and Carbohydrate Profiles, Rumen Degradation and Intestinal Digestion Characteristics, and Predicted Truly Absorbed Protein Supply

Protein structural profiles have been applied to predict true protein supply of different feeds (Liu et al., 2013; Theodoridou and Yu, 2013a); however, Huang (2015) did not find any protein-related spectral variable left in the model for predicting metabolizable protein of canola meal that pelleted at different conditions. This suggests that carbohydrate structural variables should be included in the prediction of protein metabolizable characteristics. The model was Y = STCHO 1st peak height (STCHO_H1) + STCHO 2nd peak height (STCHO_H2) + STCHO 3rd peak height (STCHO_H3) + STCHO area (STCHO_A) + cellulosic compounds height (CELC_H) + cellulosic compounds area (CELC_A) + total CHO 1st peak height (CHO_H1) + total CHO 2nd peak height (CHO_H2) + total CHO 3rd peak height (CHO_H3) + total CHO 1st peak area (CHO_A1) + total CHO 2nd peak area (CHO_A2) + total CHO 3rd peak area (CHO_A3) + total CHO area (CHO_A).

There was no carbohydrate-relative spectral variable left to predict CHO profiles for carinata seeds and canola seeds, whereas structural CHO and cellulosic compounds would be selected as predictors for protein degradation and digestion (Table 4.10). Structural CHO 3rd peak height could be used as a predictor of BCP and accounted for 59% of the variance, while CELC peak height was left in the prediction model of EDCP. The intestinal digestion of protein could be predicted by STCHO area. According to the results in the Table 4.10, peak height of cellulosic compounds (CELC_H) could be the predictor for not only total digested protein but also DVE and FMV, with 75%, 44% and 43% of the variance accounted for. The 2nd peak height of STCHO was the only variable left for OEB, accounting for 62% of the total variance. Our results proved that

carbohydrate structural characteristics of these oilseeds could be important for determining protein degradation and digestion.

4.3.4.2. Regression Study of Nutrient Structural Spectral Features and Bioavailability in Carinata and Canola Co-products

4.3.4.2.1. Regression Study of Protein Structure Spectral Features and Protein Profiles, Rumen Degradation and Intestinal Digestion Characteristics, and Predicted Truly Absorbed Protein Supply

Concerning the protein profiles, the area ratio of amide I to amide II was the only predictor of crude protein, neutral detergent insoluble crude protein (NDICP) and acid detergent insoluble crude protein (ADICP) of carinata meal, hexane-extracted carinata presscake and canola meal, accounting for 86%, 79% and 76% of total variance, respectively (Table 4.11). The variable left to predict soluble crude protein was height ratio of amide I to amide II with 78% of variance explained, while non-protein nitrogen could be predicted by height ratio of α-helix to β-sheet (71% of the variance accounted for). The predictors for CP and SCP were in accordance with the results from Liu et al. (2013) for different dried distiller grains with soluble, and Huang (2015) for pelleted canola meal.

For protein degradation in the rumen, height ratio of amide I to amide II could be used to predict rumen bypass protein for these three co-products, however the area ratio was the predictor of EDCP (75% and 78% of the variance explained respectively). Intestinal digested protein was estimated by amide I area and amide II peak height, and area ratio of amide I to amide II was used for total digested protein. However, Theodoridou and Yu (2013a) reported that protein profiles and digestion were related to protein secondary structure (height ratio of α-helix to β-sheet). The truly digested protein in the small intestine (DVE), as well as FMV^{DVE}, was predicted by amide I area and β-sheet height accounting for 99% of the variance. The area ratio of amide I to amide II was the predictor for degraded protein balance, which accounted for 79% of the total variance.

Huang (2015) found that, in heat-processed canola meal pellet, protein degradation and digestion characteristics were mostly explained by carbohydrate-related structural variables.

4.3.4.2.2. Regression Study of Carbohydrate Structure Spectral Features and Carbohydrate Profiles, Rumen Degradation and Intestinal Digestion Characteristics, and Predicted Truly Absorbed Protein Supply

The carbohydrate profiles of carinata meal, hexane-extracted carinata presscake and canola meal could be predicted by carbohydrate structural variables according to the results of our multiple regression analyses (Table 4.12). Cellulosic compounds peak height and the third peak area of total CHO were the predictors of carbohydrate, accounting for 97% of the variance. The fiber content (NDF, ADF and ADL) was explained by the second peak height of structural CHO. Moreover, rumen degradation of protein also had relationship with structural CHO, with the first peak height and the second peak height explaining BCP^{DVE} and EDCP respectively (90% and 85% of the variance was accounted for). Related to rumen bypass protein, intestinal digested protein could also be predicted by structural CHO 1^{st} peak height with 86% of variance explained. The total digested protein was predicted by structural CHO 2^{nd} peak height instead. Regarding the relationship between CHO structure and truly digested protein in the small intestine, the second peak area at total CHO region was the only predictor for DVE (84% of the variance accounted for), but OEB was predicted by the second peak height of structural CHO (85% of variance accounted for). Huang (2015) found the first peak area of total CHO was selected for rumen degraded protein, intestinal digested protein and metabolizable protein of canola meal pellet, and the second peak height of total CHO was the second predictor for metabolizable protein. This differs from our results, perhaps due to different samples, feed processing methods and models used for the regression analysis.

Table 4.9 Multiple regression analyses to find the important protein structural variables for predicting protein profiles, rumen protein degradation, intestinal protein digestion and truly absorbed protein supply of new carinata seeds (Yellow-AAC A110 and Brown-110915EM), new canola seeds (Yellow-YN07 C1386 and Brown-N07 1374) and a commercial canola seed (Brown)

Predicted variables (Y)	Variable(s) selection (Variables left in the model with P<0.05)	Prediction equation Test model: Y= a + b1 × x1 + b2 × x2…	Model R^2 value	RSD	P value
Protein profiles					
CP (%DM)	H_A left in the model	CP = 6.44 + 259.39 × H_A	0.56	2.22	0.01
NDICP (%CP)	H_A left in the model	NDICP = 14.78 – 141.55 × H_A	0.56	1.21	0.01
ADICP (%CP)	No variable left				
SCP (%CP)	No variable left				
NPN (%CP)	H_A left in the model	NPN = -42.98 + 847.95 × H_A	0.52	7.95	0.02
Rumen protein degradation					
BCP^{DVE} (g/kg DM)	A_AII left in the model	BCP^{DVE} = 33.79 + 17.76 × A_AII	0.68	3.56	0.003
EDCP (g/kg DM)	H_AI, A_AI left in the model	EDCP = 46.08 + 13040 × H_AI – 157.44 × A_AI	0.76	15.19	0.01
Intestinal protein digestion					
IDP (g/kg DM)	No variable left				
TDP (g/kg DM)	H_A left in the model	TDP = 39.06 + 2402.78 × H_A	0.55	20.97	0.01
DVE/OEB model					
DVE (g/kg DM)	No variable left				
OEB (g/kg DM)	H_AI, A_AI left in the model	OEB = 12.40 + 10376 × H_AI – 125.32 × A_AI	0.79	11.01	0.004
FMV (kg milk/kg feed)	No variable left				

Notes: Protein structure spectral parameters: H_AI: amide I peak height; A_AI: amide I area; H_AII: amide II peak height; A_AII: amide II area; H_AI_II: peak height ratio of amide I to amide II; A_AI_II: area ratio of amide I to amide II; H_A: α-helix peak height; H_B: β-sheet peak height; H_AB: peak height ratio of α-helix to β-sheet. RSD: residual standard deviation. DM: dry matter; CP: crude protein; NDICP: neutral detergent insoluble crude protein; ADICP: acid detergent insoluble crude protein; SCP: soluble crude protein; NPN: non-protein nitrogen; BCP^{DVE}: rumen bypass crude protein in DVE/OEB system; EDCP: effectively degraded crude protein; IDP: intestinal digested protein; TDP: total digested protein; DVE: truly digested protein in the small intestine; OEB: degraded protein balance; FMV^{DVE}: feed milk value in DVE/OEB system.

Table 4.10 Multiple regression analyses to find the important carbohydrate structural variables for predicting carbohydrate profiles, rumen protein degradation, intestinal protein digestion and truly absorbed protein supply of new carinata seeds (Yellow-AAC A110 and Brown-110915EM), new canola seeds (Yellow-YN07 C1386 and Brown-N07 1374) and a commercial canola seed (Brown)

Predicted variables (Y)	Variable(s) selection (Variables left in the model with P<0.05)	Prediction equation Test model: Y= a + b1 × x1 + b2 × x2…	Model R^2 value	RSD	P value
CHO profiles					
CHO (%DM)	No variable left				
NDF (%DM)	No variable left				
ADF (%DM)	No variable left				
ADL (%DM)	No variable left				
Rumen protein degradation					
BCP^{DVE} (g/kg DM)	STCHO_H3 left in the model	BCP^{DVE} = 48.91 + 1339.50 × STCHO_H3	0.59	4.03	0.01
EDCP (g/kg DM)	CELC_H left in the model	EDCP = 68.67 + 6765.11 × CELC_H	0.66	16.99	0.004
Intestinal protein digestion					
IDP (g/kg DM)	STCHO_A left in the model	IDP = -14.99 + 11.70 × STCHO_A	0.45	4.98	0.03
TDP (g/kg DM)	CELC_H left in the model	TDP = 80.18 + 7748.31 × CELC_H	0.75	15.65	0.001
DVE/OEB model					
DVE (g/kg DM)	CELC_H left in the model	DVE = 17.34 + 2471.81 × CELC_H	0.44	9.80	0.04
OEB (g/kg DM)	STCHO_H2 left in the model	OEB = 247.67 – 8388.98 × STCHO_H2	0.62	14.01	0.01
FMV (kg milk/kg feed)	CELC_H left in the model	FMV^{DVE} = 0.35 + 49.96 × CELC_H	0.43	0.20	0.04

Notes: Carbohydrate structure spectral parameters: STCHO_H1: 1st peak height of STCHO; STCHO_H2: 2nd peak height of STCHO; STCHO_H3: 3rd peak height of STCHO; STCHO_A: STCHO area; CELC_H: CELC peak height; CELC_A: CELC area; CHO_H1: 1st peak height of total CHO; CHO_H2: 2nd peak height of total CHO; CHO_H3: 3rd peak height of total CHO; CHO_A1: 1st peak area of total CHO; CHO_A2: 2nd peak area of total CHO; CHO_A3: 3rd peak area of total CHO; CHO_A: total CHO area. RSD: residual standard deviation. CHO: carbohydrate; STCHO: structural CHO; CELC: cellulosic compounds; NDF: neutral detergent fiber; ADF: acid detergent fiber; ADL: acid detergent lignin; BCP^{DVE}: rumen bypass crude protein in DVE/OEB system; EDCP: effectively degraded crude protein; IDP: intestinal digested protein; TDP: total digested protein; DVE: truly digested protein in the small intestine; OEB: degraded protein balance; FMV^{DVE}: feed milk value in DVE/OEB system.

Table 4.11 Multiple regression analyses to find the important protein structural variables for predicting protein profiles, rumen protein degradation, intestinal protein digestion and truly absorbed protein supply of carinata meal, hexane-extracted carinata presscake and canola meal

Predicted variables (Y)	Variable(s) selection (Variables left in the model with P<0.05)	Prediction equation Test model: Y= a + b1 × x1 + b2 × x2…	Model R^2 value	RSD	P value
Protein profiles					
CP (%DM)	A_AI_II left in the model	CP = 257.16 – 86.58 × A_AI_II	0.86	2.84	0.003
NDICP (%CP)	A_AI_II left in the model	NDICP = -100.63 + 44.16 × A_AI_II	0.79	1.84	0.01
ADICP (%CP)	A_AI_II left in the model	ADICP = -41.11 + 17.78 × A_AI_II	0.76	0.81	0.01
SCP (%CP)	H_AI_II left in the model	SCP = -448.67 + 291.74 × H_AI_II	0.78	11.27	0.01
NPN (%CP)	H_AB left in the model	NPN = -109.81 + 125.19 × H_AB	0.71	4.82	0.02
Rumen protein degradation					
BCP^{DVE} (g/kg DM)	H_AI_II left in the model	BCP^{DVE} = 1296.46 – 686.82 × H_AI_II	0.75	28.91	0.01
EDCP (g/kg DM)	A_AI_II left in the model	EDCP = 3687.01 – 1370.80 × A_AI_II	0.78	58.36	0.01
Intestinal protein digestion					
IDP (g/kg DM)	A_AI, H_AII left in the model	IDP = -127.45 + 234.24 × A_AI – 25380 × H_AII	0.95	8.70	0.002
TDP (g/kg DM)	A_AI_II left in the model	TDP = 2932.71 – 1033.83 × A_AI_II	0.84	35.98	0.003
DVE/OEB model					
DVE (g/kg DM)	A_AI, H_B left in the model	DVE = 34.08 + 109.11 × A_AI – 7714.66 × H_B	0.99	2.01	<0.001
OEB (g/kg DM)	A_AI_II left in the model	OEB = 3179.98 – 1208.56 × A_AI_II	0.79	50.58	0.01
FMV (kg milk/kg feed)	A_AI, H_B left in the model	FMV^{DVE} = 0.70 + 2.22 × A_AI – 157.01 × H_B	0.99	0.04	<0.001

Notes: Protein structure spectral parameters: H_AI: amide I peak height; A_AI: amide I area; H_AII: amide II peak height; A_AII: amide II area; H_AI_II: peak height ratio of amide I to amide II; A_AI_II: area ratio of amide I to amide II; H_A: α-helix peak height; H_B: β-sheet peak height; H_AB: peak height ratio of α-helix to β-sheet. RSD: residual standard deviation. DM: dry matter; CP: crude protein; NDICP: neutral detergent insoluble crude protein; ADICP: acid detergent insoluble crude protein; SCP: soluble crude protein; NPN: non-protein nitrogen; BCP^{DVE}: rumen bypass crude protein in DVE/OEB system; EDCP: effectively degraded crude protein; IDP: intestinal digested protein; TDP: total digested protein; DVE: truly digested protein in the small intestine; OEB: degraded protein balance; FMV^{DVE}: feed milk value in DVE/OEB system.

Table 4.12 Multiple regression analyses to find the important carbohydrate structural variables for predicting carbohydrate profiles, rumen protein degradation, intestinal protein digestion and truly absorbed protein supply of carinata meal, hexane-extracted carinata presscake and canola meal

Predicted variables (Y)	Variable(s) selection (Variables left in the model with P<0.05)	Prediction equation Test model: Y= a + b1 × x1 + b2 × x2…	Model R^2 value	RSD	P value
CHO profiles					
CHO (%DM)	CELC_H, CHO_A3 left in the model	CHO = 59.15 – 3442.22 × CELC_H + 3.56 × CHO_A3	0.97	1.43	0.001
NDF (%DM)	STCHO_H2 left in the model	NDF = 2.97 + 1999.75 × STCHO_H2	0.89	2.70	0.001
ADF (%DM)	STCHO_H2 left in the model	ADF = 0.86 + 1622.03 × STCHO_H2	0.93	1.75	0.001
ADL (%DM)	STCHO_H2 left in the model	ADL = -2.94 + 1034.68 × STCHO_H2	0.93	1.09	<0.001
Rumen protein degradation					
BCP^{DVE} (g/kg DM)	STCHO_H1 left in the model	BCP^{DVE} = -39.25 + 13236 × STCHO_H1	0.90	18.27	0.001
EDCP (g/kg DM)	STCHO_H2 left in the model	EDCP = 572.91 – 30208 × STCHO_H2	0.85	49.16	0.003
Intestinal protein digestion					
IDP (g/kg DM)	STCHO_H1 left in the model	IDP = -34.95 + 8075.99 × STCHO_H1	0.86	13.69	0.003
TDP (g/kg DM)	STCHO_H2 left in the model	TDP = 577.59 – 21774 × STCHO_H2	0.83	37.29	0.004
DVE/OEB model					
DVE (g/kg DM)	CHO_A2 left in the model	DVE = 68.53 + 43.36 × CHO_A2	0.84	9.00	0.004
OEB (g/kg DM)	STCHO_H2 left in the model	OEB = 433.94 – 26556 × STCHO_H2	0.85	43.00	0.003
FMV (kg milk/kg feed)	CHO_A2 left in the model	FMV^{DVE} = 1.40 + 0.88 × CHO_A2	0.84	0.18	0.004

Notes: Carbohydrate structure spectral parameters: STCHO_H1: 1st peak height of STCHO; STCHO_H2: 2nd peak height of STCHO; STCHO_H3: 3rd peak height of STCHO; STCHO_A: STCHO area; CELC_H: CELC peak height; CELC_A: CELC area; CHO_H1: 1st peak height of total CHO; CHO_H2: 2nd peak height of total CHO; CHO_H3: 3rd peak height of total CHO; CHO_A1: 1st peak area of total CHO; CHO_A2: 2nd peak area of total CHO; CHO_A3: 3rd peak area of total CHO; CHO_A: total CHO area. RSD: residual standard deviation. CHO: carbohydrate; STCHO: structural CHO; CELC: cellulosic compounds; NDF: neutral detergent fiber; ADF: acid detergent fiber; ADL: acid detergent lignin; BCP^{DVE}: rumen bypass crude protein in DVE/OEB system; EDCP: effectively degraded crude protein; IDP: intestinal digested protein; TDP: total digested protein; DVE: truly digested protein in the small intestine; OEB: degraded protein balance; FMV^{DVE}: feed milk value in DVE/OEB system.

4.4. Conclusions

The inherent structural characteristics of new carinata seeds, canola seeds and three seed co-products can be detected by ATR-FTIR. The univariate spectral analysis showed differences in absorption intensities (peak heights and areas) of functional groups related to protein and carbohydrate. The results of multivariate spectral analysis showed similar protein and carbohydrate structure within the whole region data involved, except differences were found between hexane-extracted carinata presscake and canola meal. The band intensities of each functional group reflect only partial information (peaks) about molecular structure at specific regions, while the data for multivariate spectral analysis (PCA and CLA) cover the entire spectral region. Based on the combination of results from univariate and multivariate analysis, there were some differences between carinata seeds and canola seeds in protein and CHO structure spectral characteristics but these were not distinguishable in CLA and PCA plots. Carinata meal had different protein and CHO structural profiles to canola meal, but showed similarities based on multivariate statistical analysis. Cold pressing in this study caused different structural features to carinata compared with commercial crushing (such as peak height and area characteristics of protein and CHO related functional groups), but did not alter the entire spectral region of functional groups significantly based on CLA and PCA results.

As for the correlation study, both protein and carbohydrate spectral profiles were correlated with protein degradation and digestion characteristics for carinata and canola seeds and co-products. The results of the multiple regression study proved that both protein and carbohydrate structural variables could be used for the prediction of rumen protein degradation kinetics, protein intestinal digestion features and protein supply for dairy cows, because carbohydrate-relative structural features were related to protein metabolism and utilization.

5. GENERAL DISCUSSION, CONCLUSIONS AND FUTURE RESEARCH

Brassica carinata is a productive oil crop for biofuel industry in semi-arid areas with good heat and drought tolerance (Rakow and Getinet, 1998). The breeding and seed quality studies of *Brassica carinata* have been conducted in Agriculture and Agri-Food Canada (AAFC). It has high crude protein and oil, but low fiber, which make it sufficient to be a potential energy source in animal feed. The co-products from biofuel processing, carinata meal and hexane-extracted carinata presscake, with high crude protein and low fiber, would be a superior protein source added to the animal rations. However, the nutritional values and bioavailability of new lines of *Brassica carinata* and carinata co-products have not been clear enough for dairy ration and industrial application. This study investigated the nutritional values of newly developed carinata seeds and two carinata co-products for dairy cows, in terms of chemical profiles, energy values, protein and carbohydrate fractions, rumen degradation kinetics, hourly effective degradation ratios of ED_N to ED_OM, intestinal protein digestion and predicted truly absorbed protein supply based on two systems. FTIR-ATR was used to reveal the molecular structural features of carinata seeds and co-products in order to determine molecular structural differences.

In Section 3, it was found that new carinata seeds had lower crude fat than canola seeds, while the yellow carinata seed had the highest crude protein, lowest NDF and ADL. Carinata meal was similar to the commercial canola meal in CP, NPN and NDICP, but had less fiber. The hexane-extracted carinata presscake, had less oil portion and higher soluble protein compared with carinata meal. Both carinata seed and co-products were significantly high in glucosinolates, similar to early carinata varieties (Xin et al., 2014a), indicating no improvement in the new *Brassica carinata* line for glucosinolate content. And cold pressing with hexane extraction did not effectively decrease glucosinolate. The high glucosinolate content may reduce feed intake, impair growth and health

of cows if adding carinata products into dairy rations. Thus, to keep glucosinolate content less than 11 μmol/g diet as Tripathi and Mishra (2007) suggested, effective methods should be applied to carinata products, for example heating, extrusion, microbial fermentation, copper sulfate treatment, or breeding low-glucosinolate carinata lines. Canola seed provides energy for animals when used in the feed, with yellow carinata seed having similar energy value compared to yellow canola seed, which is obviously higher than that of the brown seed. Carinata meal was similar to canola meal in energy supply, and the hexane-extracted carinata presscake with lower fat had the similar energy value to carinata meal.

As for rumen degradation kinetics, carinata seeds had high rumen-degraded organic matter and crude protein, providing high energy and N for microbial activity. Furthermore, carinata seeds had more rumen undegraded protein passed on to the small intestine. The two carinata co-products had more effectively degraded OM and CP than canola meal, but canola meal was significantly higher in RUP and EDNDF. A higher RDP would result in more nitrogen supply to the rumen, but with limited energy, the redundant N cannot be utilized for microbial protein synthesis and would potentially be N loss. According to the hourly effective degradation ratios between N and OM (ED_N/ED_OM), all seeds had degradation ratios above the optimal N to OM ratio (25g N/kg OM) in the beginning (Figure 3.1). However, at 24 h of rumen incubation, only yellow carinata seed was over the optimal ratio, which showed there were still available N to utilize based on available energy. Carinata co-products had higher ED_N/ED_OM ratios primarily when incubated in the rumen, but their ratios declined faster than canola meal during the rumen degradation. The ratio of hexane-extracted carinata presscake was less than the optimal after 18 hours of rumen degradation, which indicated N deficiency (Figure 3.2). New carinata seeds could offer similar intestinal digested CP compared with new canola seeds, but higher than the commercial canola seed.

Compared with canola meal, carinata meal had less intestinal digested protein, and hexane-extracted carinata presscake had the least IDP. Combining the results based on the DVE/OEB system and the NRC Dairy model, carinata seeds had higher metabolizable protein than canola seeds, contributing to a higher feed milk value. This supported that carinata seeds could be a protein source with a surplus of N, compared to canola seeds. Carinata meal had similar predicted protein supply in the DVE/OEB system compared to canola meal, but less protein supply in the NRC Dairy model. The hexane-extracted carinata presscake had the lowest absorbed protein supply to dairy cows.

To estimate the nutritional value of carinata meal in a dairy ration, 3.71 %DM carinata meal was added to replace 3.71 %DM canola meal in a dairy diet (Appendix). The diet with carinata meal had higher soluble crude protein (5.3 %DM), lower aNDFom (30.8 %DM), similar NFC compared with a diet with canola meal. The two diets both met the requirements of ME and MP for a high-production dairy cow. For the predicted milk production based on ME, a lactating dairy cow could produce 42.5 kg milk, relatively higher than milk production if fed a diet with canola meal (42.0 kg). A lactating cow could produce 44.6 kg milk potentially based on metabolizable protein supply of a diet with carinata meal, but may produce 43.2 kg milk if fed the diet with canola meal. This may indicate that carinata meal could be implied as a high protein source for dairy rations. The two diets with carinata meal and canola meal respectively could provide NE_L about 1.6 Mcal/kg feed for lactating cows.

The molecular structural features were estimated in Section 4, to know the structural differences of carinata seeds vs. canola seeds, and carinata co-products vs. canola meal. The univariate spectral analysis showed that new carinata seeds had significantly different protein and CHO structural spectral features from canola seeds, but structural differences were not fully

distinguished between new carinata seeds and canola seeds by multivariate analyses as mixed clusters and ellipses shown in the CLA and PCA plots. It means that carinata seeds and canola seeds had unique spectral features on individual functional group regions, but showed similarity on the entire molecular structural spectral region. Seeds with different hull colors (yellow or brown) were similar to each other in most protein and CHO structural profiles. For three co-products, carinata meal had the most similar protein structural characteristics with canola meal, while the carbohydrate structure profiles were different ($P<0.05$). The multivariate analyses results did not show any distinction between carinata and canola meal, carinata meal and hexane-extracted carinata presscake in either protein or carbohydrate structure considering the whole spectral region. Only the hexane-extracted carinata presscake was found significantly different from canola meal within the protein and CHO structural regions. To reveal the relationships between molecular structural spectral parameters and nutritive bioavailability, correlation and regression studies were conducted. Protein and carbohydrate spectral profiles were correlated with protein degradation and intestinal digestion characteristics of carinata seeds and co-products. It was then possible to select carbohydrate and protein structural variables to predict rumen degradation and intestinal digestion characteristics of protein, as well as protein supply of carinata seeds and carinata co-products. For example, for oil seeds, EDCP could be predicted by 46.08 + 13040 × amide I peak height - 157.44 × amide I area ($R^2=0.76$). Another important nutritional value, truly digested protein in the small intestine (DVE), could be estimated as 68.53 + 43.36 × total CHO 2^{nd} peak area ($R^2=0.84$) for carinata co-products.

In conclusion, carinata seeds had different chemical composition and molecular structural characteristics compared with canola seeds. Specifically, carinata was high in protein but low in fiber, could offer similar energy to dairy cows compared with canola seed. Concerning protein

metabolism if fed to dairy cows, carinata seeds were observed to have higher ruminal CP and OM degradation and total digested protein supply, but lower intestinal digested protein. In accordance to the predicted truly absorbed protein supply, carinata seeds could be a good energy and protein source for dairy diets, of which the yellow seed would be regarded as a better feed source than the brown. The co-product from biofuel processing, carinata meal, showed higher protein and energy supply than canola meal. Considering its digestion features, it could be a superior protein source for lactating dairy cows compared to canola meal, with a higher predicted milk yield. The hexane-extracted carinata presscake showed advantage in energy supply and nutrient solubility, however with higher nutrient solubility, the intestinal digestible protein was inhibited and thus protein supply for milk production negatively affected compared with carinata meal. To summarize, newly developed carinata seeds and their co-products could be alternative feed sources for dairy rations, but processing needs to be conducted to reduce glucosinolates while adding to the diets. With protein and carbohydrate structure detected by FTIR, spectral profiles can be used as predictors to estimate protein digestion characteristics and predict protein supply of *Brassica carinata* and its co-products, which may help to save time and expense of conducting animal feeding trials.

This study reveals nutritional quality of the newly developed carinata seeds and carinata co-products, which contributes to the implication and feed registration data of carinata products. These findings may also be helpful to plant breeders to improve seed quality of *Brassica carinata* and to develop low-glucosinolate *Brassica carinata* lines. Our results showed high glucosinolates in carinata products, which might not be beneficial for herd health. Several effective processing methods can be applied to carinata seed and meal while added to animal diets, such as heating, pelleting or extrusion. Further studies could focus on feeding behaviors, amino acid profiles and flow, milk production and composition, and nutritional value of dairy diets with different

percentages of carinata products. Moreover, various feed processes (for example heating and pelleting, with different conditioning time and temperatures) could be investigated to reach the optimal nutritional value of carinata meal. FTIR-ATR, as a rapid and accurate technique of detecting inherent molecular structure features, can be applied by feed industry to determine the effects of feed processing and nutritional values of different feedstuff, and for prediction of nutrient bioavailability in animals. It is also suggested to build a data deck of molecular structure and nutrient metabolism information for different feed types and eventually find universal predictors from rapidly-obtained spectral variables for nutritional characteristics.

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7. APPENDIX

Ingredient composition of diets for lactating dairy cows

	Diet[1]	
Ingredient, %DM	Carinata Meal	Canola Meal
Corn silage	35.28	35.28
Alfalfa hay	16.71	16.71
Parlour concentrate[2]	44.27	44.27
Carinata meal	3.71	-
Canola meal solvent	-	3.71
Water	0.02	0.02

[1] Diet: dairy diets with carinata meal or canola meal respectively.

[2] Parlour concentrate contained 45.81% barley grain, 17.56% corn grain, 7.38% pea grain, 7.45% canola meal, 8.21% soybean meal, 1.90% corn gluten meal, 3.22% corn dist medium spirits, 2.38% U of S Premix (16% Ca, 7% P, 7% Mg, 2% K, 10% Cl, 1.25% S, 1507 ppm Mn, 678 ppm Cu, 1005 ppm Fe, 2513 ppm Zn, 80 ppm I, 30 ppm Co, 20 ppm Se, 251 256 IU/kg Vit A, 80 402 IU/kg Vit. D3, 2010 IU/kg Vit E), 1.39% PR10 palmitic, 1.45% Molasses cane, 0.07% biotin (2% biotin source), 0.42% R-choline (25% choline source), 0.16% PotMagSulfate, 1.01% sodium bicarbonate, 1.08% limestone, 0.04% niacin, 0.02% santoquin, 0.44% salt.

Nutrient digestibility and supply to dairy cows (based on CNCPS 6.5[1])

	Diet[2]	
Item[3]	Carinata Meal	Canola Meal
CP (%DM)	15.7	15.4
SCP (%DM)	5.3	4.6
aNDFom (%DM)	30.8	31.2
NFC (%DM)	42.5	42.4
Sugar (%DM)	3.7	3.8
Starch (%DM)	28.7	28.7
Soluble fiber (%DM)	8.0	7.9
EE (%DM)	3.5	3.5
ME (%Req)	104.6	103.7
Milk_ME (kg)	42.5	42.0
MP (%Req)	107.6	105.3
Milk_MP (kg)	44.6	43.2
NE_L (Mcal/kg)	1.6	1.6

[1] Diets analysed according to CNCPS 6.5 by NDS software (Ruminant Management & Nutrition, Reggio Emilia, Italy).

[2] Diet: dairy diets with carinata meal or canola meal respectively.

3 CP: crude protein; DM: dry matter; SCP: soluble crude protein; aNDFom: neutral detergent fiber corrected by sodium sulfate and ash; NFC: non-fiber carbohydrate; EE: crude fat; ME: metabolizable energy; Req: requirement of dairy cows; Milk_ME: milk production based on ME; MP: metabolizable protein; Milk_MP: milk production based on MP.